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The epidemiology and clinical outcomes associated with *Theileria parva* in a cohort of East African short horn zebu calves

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"You know, farming looks mighty easy when your plow is a pencil and you're a thousand miles from the corn field."

Dwight D. Eisenhower, September 11, 1956

Declaration

This dissertation is submitted to the University of Edinburgh in accordance with the requirements for the degree of Doctor of Philosophy in the faculty of Medicine and Veterinary Medicine. Some of the data described in this thesis was not collected by me. The details of when this was the case are presented below. In each case, the analysis of this data was my own work.

Serological testing

This testing was carried out by Dr. Henry Kiara, Alice Njeri, and Dickens Odhiambo Okomo at the International Livestock Research Institute, Nairobi, Kenya

Reverse line blot testing

This testing was carried out by Evalyne Njiiri at the Onderstepoort Veterinary Research Institute, the University of Pretoria, South Africa.

Preparation and histological examination of tissues collected post-mortem

This testing was carried out by the pathology department at the Onderstepoort Veterinary Research Institute, the University of Pretoria, South Africa.

Extraction of DNA, preparation of samples, and analysis of SNP data

This work was carried out by Dr. Mary Ndila at ILRI, in association with Dr. Miika Tapio (MTT Agrifood research, Finland). and Prof. Olivier Hanotte (Nottingham University).

Field data

Dr. Olga Tosas-Auguet, Dr. Maagai Kaare, Katherine Allen, Dr. Samuel Thumbi, and

myself were responsible for the management of data collection in the field at different times during the 3 years of data collection.

Signed:

Amy Jennings

Thesis abstract

This thesis takes data from the Infectious Diseases of East African Livestock (IDEAL) project. The project was a longitudinal calf cohort study based in Western Kenya. Indigenous short horn zebu calves were recruited at birth and then visited every 5 weeks through their first year of life.

The aim of this thesis was to improve understanding of the epidemiology of *Theileria parva*, with a particular focus on variation in host response. 362 of the 548 calves in the study cohort were classified as having seroconverted to *T. parva*, and 381 to *T. mutans* before 1 year old. The diagnostic tools used to identify exposure in the calf were compared, and environmental and calf level risk factors associated with the age at seroconversion were sought. Decreased elevation of the homestead and increased size of the herd were found to be significantly associated with an increased hazard of seroconversion to *T. parva*. There was little variation in hazard of *T. mutans* captured across the study site.

The outcome *'clinical episode'* was used to classify whether the calf was ill at each routine visit. A large number of calves passed through their first year of life without clinical disease being observed, and a minority of calves experienced the majority of clinical episodes. Multiple clinical episodes were apparently related in time, suggesting that they were due either to the same or connected pathogenic processes. A low birth weight, larger herds, and older farmers were all risk factors for being a sick calf. Both high helminth burden and *T. parva* were found to be significantly associated with clinical disease at a population level.

A lot of variation was seen in the clinical presentation of disease. The clinical signs associated with fatal East Coast Fever (ECF), the clinical disease associated with *T.*

parva infection, were found to be very variable. Although this may have been partly due to the varying times in the disease process that calves were observed prior to death, the complication of the clinical picture was also suggested to be due to co-infections.

71% of the cohort was infected with *T. parva* in their first year of life, but only a fraction (8.7%) went on to die from that infection. Unmatched and matched nested case control study formats were used to investigate the risk factors associated with death following *T. parva* infection (ECF death) in these calves. It was found that being infected young was a risk factor for death. Calves owned by older farmers were also at higher risk of death following infection. Going out grazing was found to be protective, and equivocal evidence was found for an association between prior *T. mutans* exposure and reduced odds of ECF death. If these initial findings from this work are correct, it is likely that *T. mutans* is influencing the clinical presentation of *T. parva* in endemic regions.

Acknowledgments

The IDEAL project was most definitely a team effort, and each member has made contributions to this thesis and my development that they will most likely never know or be specifically thanked for. So to the wide and varied IDEAL team - thank you.

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Finally thank you to my family, direct and extended. Mum and Dad, you have a wrinkle for every airport good bye. And my Matthew, we did this together, and here's to the next adventure.

Publications

The following papers include work that has arisen from this thesis.

- *A live weight-heart girth relationship for accurate dosing of east African shorthorn zebu cattle*

  Maia Lesosky, Sarah Dumas, Ilana Conradie, Ian Graham Handel, Amy Jennings, Samuel Thumbi, Phillip Toye, Barend Mark de Clare Bronsvoort

  Tropical Animal Health and Production, 2012, 1:6

- *Design and descriptive epidemiology of the Infectious Diseases of East African Livestock (IDEAL) project, a longitudinal calf cohort study in western Kenya*

  Barend Mark de Clare Bronsvoort, Samuel Mwangi Thumbi, Elizabeth Jane Poole, Henry Kiara, Olga Tosas Auguet, Ian Graham Handel, Amy Jennings, Ilana Conradie, Mary Ndila Mbole-Kariuki, Philip G Toye, Olivier Hanotte, JAW Coetzer and Mark EJ Woolhouse

  BMC Veterinary Research 2013, 9:171

- *Maternal antibody uptake, duration and influence on survival and growth rate in a cohort of indigenous calves in a smallholder farming system in western Kenya*

  Philip Toye, Ian Handel, Julia Gray, Henry Kiara, Samuel Thumbi, Amy Jennings, Ilana Conradie van Wyk, Mary Ndila, Olivier Hanotte, Koos Coetzer, Mark Woolhouse, Mark Bronsvoort

  Veterinary Immunology and Immunopathology, Volume 155, Issues 1 2, September 2013, 129:134

Contents

Table of abbreviations

AEZ	Agro-ecological zone
AHA	Animal health assistant
AUC	Area under the curve (ROC)
DNA	Deoxyribonucleic acid
ECF	East coast fever
ELISA	Enzyme-linked immunosorbent assay
ID	Identification (number)
IDEAL	Infectious diseases of east African livestock
ILRI	International livestock research institute
LAHA	Local animal health assistant
LCL	Lower confidence limit on 95% confidence interval
LM	Lower middle AEZ
LYM	Lymphocyte
NGO	Non-governmental organisation
PCV	Packed cell volume (erythrocytes)
PCR	Polymerase chain reaction
PP	Percentage positivity of the optical density of the positive control (ELISA)
REF	Reference category (categorical variable in regression)
RLB	Reverse line blot
ROC	Receiver operator characteristic (curve)
RT	Rectal temperature
SHZ	East African short horn zebu
SL	Sublocation
TDHA	Time-discrete hazard analysis
TLU	Tropical livestock units

TP Total serum protein

UCL Upper confidence limit on 95% confidence interval

UM Upper middle AEZ

WBC White blood cell

Chapter 1

Introduction

1.1 The IDEAL project

1.1.1 Key aims and objectives

The Infectious Diseases of East African Livestock (IDEAL) project was a multi-disciplinary study conceived and designed to address three major issues:

- To improve the understanding of the epidemiology of infectious diseases affecting cattle in tropical regions.
- To investigate the interactions between the infections that co-infect hosts, and the effect that these interactions have on host outcome, whether these be neutral, synergystic, or antagonistic.
- To investigate whether positive traits cluster within individuals, and whether this can be exploited to improve survival or production through genetic selection.

The project was a longitudinal calf cohort study based in Western Kenya. East African short horn zebu calves were recruited at birth and then visited every 5 weeks through their first year of life. Their growth, mortality, and clinical episodes were recorded. At each visit a detailed clinical history and examination were carried out, and samples were collected to carry out routine haematology and parasitological testing. Samples

were also placed in a biobank, and additional laboratory diagnostic tests were carried out. This data allowed the association of a number of outcomes (growth, mortality, anaemia, clinical disease) with potential predictors.

The project was based in Busia, Kenya, a border town neighbouring Uganda. Busia is located on the main road that goes between Mombasa on the Kenyan coast and Kampala, the capital of Uganda.

1.1.2 The Kenyan small-holder mixed species farming system, and the short horn zebu

Livestock are kept widely by the rural poor, the poorest keeping chickens only, with increasing wealth allowing goats, sheep, pigs and finally cattle (Hanotte et al. 2010). These species are often kept together and are either tethered in the homestead, are free to roam, or are communally herded on common grazing. Cattle are considered by their owners to be the most valuable livestock species held in the homestead, providing milk, meat, draught power, fertiliser, building materials, status and a store of wealth (Mcdermott et al. 2004; Randolph et al. 2007; Amimo et al. 2011). However, they make for a high risk investment strategy as numerous threats face herds; infectious disease, limited access to veterinary expertise and resources, and unpredictable weather. Efficient livestock farming, sensitive to cultural expectations, can offer a tangible way for the rural poor to climb from poverty, providing a means of cash generation to support the household, improve nutrition, buy health care services, and pay for education (Perry and Grace 2009; Rich and Perry 2011; Kristjanson et al. 2004).

The main cattle breed kept in this region is the East African short horn zebu (SHZ). The short horn zebu is a genetically admixed breed with its origins in both the humped cattle domesticated in the Near East, *Bos indicus*, and the African *Bos taurus* thought to have been domesticated in the eastern part of Sahara (Hanotte et al. 2002). This breed grows slowly and has a relatively low milk yield compared to exotic breeds such as Holsteins or Fresians (1L per cow per day was measured in Busia district by Machila et al. (2003)). However, the breed is well adapted to its range and one of its

major advantages is its ability to survive in an environment heavily infested with potentially fatal infectious diseases. The breed is thought to have resistance or tolerance to several endemic diseases (Hanotte et al. 2010).

Some 'improvement' of cattle in Kenya has occurred with the import of more productive European cattle breeds. In some areas, high yielding dairy cattle, and their crosses with indigenous breeds, are successfully farmed using zero-grazing, and intensive tick management. The cattle are mainly in small-holder dairy farms, but there are also some large commercial herds. However, exotic breeds remain incredibly susceptible to disease, and they remain a rarity, especially around the IDEAL study site.

1.1.3 Western Kenya; its agriculture and veterinary infrastructure

Western Kenya borders the north eastern side of Lake Victoria and the eastern Ugandan border. It is a varied region containing Mount Elgon, the second highest peak in Kenya, and Kakamega, Kenya's last area of rainforest.

Although still very rural, Western Kenya has a high population density, a large proportion of whom are poor (>40% live below the poverty line) (Thornton et al. 2007). Many still rely on subsistence farming, although some small scale cash cropping is carried out, mainly of sugar cane and tobacco. The main subsistence crops are maize and cassava, and for those living on the lake shores, fishing offers a source of both food and income.

In the northern parts of the province higher elevations are associated with cooler temperatures. This has allowed a few small-holder dairy farmers to keep exotic cattle breeds and their crosses, usually under zero-grazing conditions. However, further south at lower elevations and closer to the lake, indigenous cattle are more commonly kept and are usually managed extensively.

The region usually experiences two fairly indistinct rainy seasons (October to December and March to May). Most farming still relies on natural rain fall rather than

irrigation and the planting and cultivation of crops follows the rains.

The region is endemic for several vector borne diseases including the tick borne diseases (theileriosis, babesiosis, anaplasmosis, and heartwater), and the tsetse borne disease, trypanosomiasis (both zoonotic and non-zoonotic species) (Barnett 1957; Latib et al. 1995; Magona et al. 2008; Bronsvoort et al. 2010). Recent activity aimed at tsetse reduction and community education has greatly reduced the burden of trypanosomiasis (FITCA 2005), but tick borne diseases continue to place a burden on livestock owners. Helminths are ubiquitous, and haemonchosis is a common problem.

Each district in Western Kenya has a government veterinary office in which a government veterinarian and other para-professionals are employed. The Department of Veterinary Services is part of the Department of Livestock Development, and is involved in monitoring and recording livestock movements and trade, in implementing national campaigns for animal health, and in offering local veterinary advice. Since 1992 veterinary services in Kenya have become increasingly privatised. This, and underfunding of government veterinary provision has led to limited veterinary services in Western Kenya, particularly in rural areas away from regions of high productivity. Farmers rarely buy veterinary pharmaceuticals and advice. Machila et al. (2003) found that in Busia District, advice and information on cattle disease management was sought from animal health assistants (AHAs) (46.3%), cattle owners and fellow farmers (39.9%) and Agrovets (11.8%). Machila et al. (2003) also found that farmers were often self diagnosing their animals' ill health and frequently doing this incorrectly. In an investigation of supply of veterinary drugs in Busia district, it was found that people supplying drugs often had no formal training in animal health (Bett et al. 2004). Therefore, even when advice is sought it is likely that advice may be inappropriate.

To summarise, Western Kenya offers an excellent setting for the investigation of the interaction between infectious disease and host outcome. This is a region where many vector borne diseases, haemoparasites, helminths, viral, and bacterial diseases are considered to be endemic. Farming is generally very low input where both reactive and prophylactic use of veterinary pharmaceuticals is a rarity. The low levels of intervention and the large number of endemic diseases present in Western Kenya offers an excellent system for investigating the interactions between a host, its environment,

and its infectious diseases. This combined with a sedentary farming system and incredibly accommodating farmers allows detailed longitudinal data to be regularly and reliably collected. Farming is a major source of income, and a means of survival for many in this region, with cattle as a key part of the production system. An improved understanding of the epidemiology and impacts of infectious disease on cattle in this region will help stakeholders to make informed decision and implement productive change to improve the lives of Kenyan cattle and their owners.

1.2 *Theileria parva*

This thesis focuses on *Theileria parva*, a potentially fatal tick-borne disease of cattle endemic to many areas of eastern Africa. East Coast fever (ECF), the clinical disease associated with *T. parva*, acts as a major limitation on the introduction of exotic breeds to this region, but also has a major financial impact on farmers keeping indigenous breeds in endemic areas.

Theileria parva is a tick-borne protozoa of the phylum Apicomplexa. This phylum also contains *Babesia*, *Plasmodium*, and the Coccidians. *T. parva* is believed to have originated in buffalo, but there are now strains circulating in domestic cattle that are adapted to that species and buffalo are not needed to maintain the parasite. *Theileria parva* is one of two *Theileria* species that are thought to be of clinical importance in cattle. The other is *T. annulata* which is found north of the Sahara. *T. parva* is endemic to many regions of East Africa, where humid and warm equatorial conditions allow its tick vector, *Rhipicephalus appendiculatus*, to breed all year round (Coetzer and Tustin 2004).

The aim of this thesis was to improve understanding of the epidemiology of *T. parva*, with a particular focus on variation in host response. This parasite offers an excellent model for investigation of variation in host response following infection as, in the IDEAL study region, *T. parva* infected the majority of animals at a young age and caused a wide range of clinical outcomes.

1.2.1 The impact of *Theileria parva* on small-holder farms

Losses due to ECF were calculated at US$168 million annually in 1989 (the most recent estimation) (Mukhebi et al. 1992) and an estimated 1 million cattle are believed to die from the disease every year (Dobbelaere and Heussler 1999) with most losses seen in calves. *T. parva* is endemic in the cattle herds of Western Province and the region was defined as an East Coast fever 'dirty area' during colonial times. Western Province cattle became highly valued due to a reputation for innate or developed resistance to the disease, and were sometimes branded with a 'T' when being traded (Norval et al. 1992). However, indigenous cattle do suffer from East Coast fever and the disease is a burden on small holder farmers, and is a significant cause of morbidity.

Investigators have sought to quantify the production losses incurred by small holder subsistence farmers in East Africa from both cause specific and non-specific mortality and morbidity. Studies investigating calf mortality in extensive small holder husbandry systems in areas close to that of the IDEAL study site reported varying death rates (from 7% (Gitau et al. 1999) to 29% (Barnett 1957)). Amongst this variation however, almost all of these studies reported *Theilieria parva* to be a highly prevalent if not the most common and consistent cause of reported deaths (Barnett 1957; Moll et al. 1984; Latib et al. 1995; Gitau et al. 1999; Swai et al. 2009).

Barnett (1957) observed calves in the Nyanza district of Kenya (a neighbouring district to that containing the IDEAL study site) from birth up until 2 years old. 845 calves in 2 areas were observed intensively for changes in their clinical state and samples were taken regularly. 29% of the calves died before they reached 2 years old and an average of 26% of these deaths were due to East Coast Fever (8% rate of ECF death), all of which happened within the first 12 months of life.

Moll et al. (1984) reported an ECF death rate in calves in the Mara district of 2.5% (total death rate by 6 months old of 19% with starvation being the highest cause of mortality) and in 1999 the importance of ECF as a cause of death in Central Kenya was confirmed (Gitau et al. 1999) with a reported death rate of 5% in open-grazed calves up to 6 months old (total mortality was 7% by 6 months old). Slightly further afield in Tanzania, Swai et al. (2009) reported a 1 year mortality rate in calves of 12%, of which 56% were attributed to tick-borne disease (38% ECF and 18% anaplasmosis).

The quantification of clinical disease has also been attempted by some authors. Barnett (1957) reported that 10-13% of calves showed clinical signs of ECF and survived that infection, Moll et al. (1984) reported 100% of calves to show some clinical signs associated with *T.parva*. Swai et al. (2009) reported 8% of calves to have experienced at least one episode of clinical disease in their first year of life, of which 43% were attributed to ECF.

ECF mainly effects calves, with most deaths occurring before one year old (Barnett 1957; Moll et al. 1984; Gitau et al. 1999; Swai et al. 2009). For farmers that often keep very few breeding animals, every calf death can mean a large proportion of annual income lost. Not only does it deprive the farmer of the potential income from the calf (either through live sale, through its potential as a draft or breeding animal, or through the meat or milk it will provide), but farmers report that, unlike exotic breeds who are separated their calf at birth and continue to milk well, short horn zebu dam milk production drops significantly once a calf is removed (reports from farmers recruited to the IDEAL study).

1.2.2 The lifecycle of *Theileria parva*

For a summary of the lifecycle of *T. parva* see figure 1.1. The vector of *T. parva* is *Rhipicephalus appendiculatus*. Ticks obtain *T. parva* infection from cattle by ingesting cattle erythrocytes infected with the piroplasm stage of the parasite. Ticks are more likely to become infected when feeding on cattle suffering from clinical East Coast Fever due to the high number of parasitised cells in the circulation at this time. However, the carrier state does exist in cattle and these carriers remain a source of infection for the tick (Young et al. 1986).

The ticks are able to become infected as larvae, but are only able to transmit as nymphs or adults. Although ticks other than *Rhipicephalus appendiculatus* have the theoretical potential to transmit *T. parva* they do not as they do not feed on cattle at immature stages (Norval et al. 1992).

Once ingested and in the tick gut, the erythrocytes are lysed and the *T. parva* piroplasms are seen free in gut smears. Micro- and macrogametes form and syngamy

occurs leading to zygote formation. This diploid stage allows genetic recombination. The zygotes develop in the gut epithelium of the tick, eventually releasing kinetes which become incorporated in to cells in the salivary glands. Sporogyny occurs in the salivary gland acinus (a cluster of saliva producing cells) to produce sporoblasts. Rapid nuclear division of sporoblasts occurs to form many sporozoites, a process that appears to be synchronised with the start of feeding (Young et al. 1980).

T. parva sporozoites are inoculated into the bovine host with the saliva of *R. appendiculatus* usually from day three to five following attachment (Young et al. 1980; Mckeever 2006). The target cell for *T. parva* sporozoites is the bovine lymphocyte, and the sporozoite becomes incorporated into the host cell within three minutes (Norval et al. 1992). The parasite within this cell type is referred to as a schizont.

Over about 3 days schizonts accumulate in lymphoblasts. The parasite does not remain within the host cell membrane but excysts and becomes incorporated in to the cell replication apparatus. Therefore, as the bovine cell divides the replicated parasites are divided and both daughter cells are infected. The schizont induces a reversible lymphoblastogenesis, which leads to a clonal expansion of infected cells (Morrison 2007).

Infected lymphoblasts collect in the local draining lymph nodes (often the parotid or the suprascapular due to the ticks preference for feeding from the ears of the host). Infected lymphoblasts metastasise via the host's circulation to other lymphoid and non-lymphoid organs. Schizonts within infected lymphoblasts start developing into merozoites 12 to 14 days following infection. The merozoites appear to bud from the surface of the schizont. This development destroys the host cell and merozoites are released. These specifically invade erythrocytes, possibly through a similar process to the penetration of sporozoites to lymphocytes. The merozoites form piroplasms within the erthyrocyte. The piroplasms are then taken up by newly attached feeding ticks and the life cycle is completed (Coetzer and Tustin 2004).

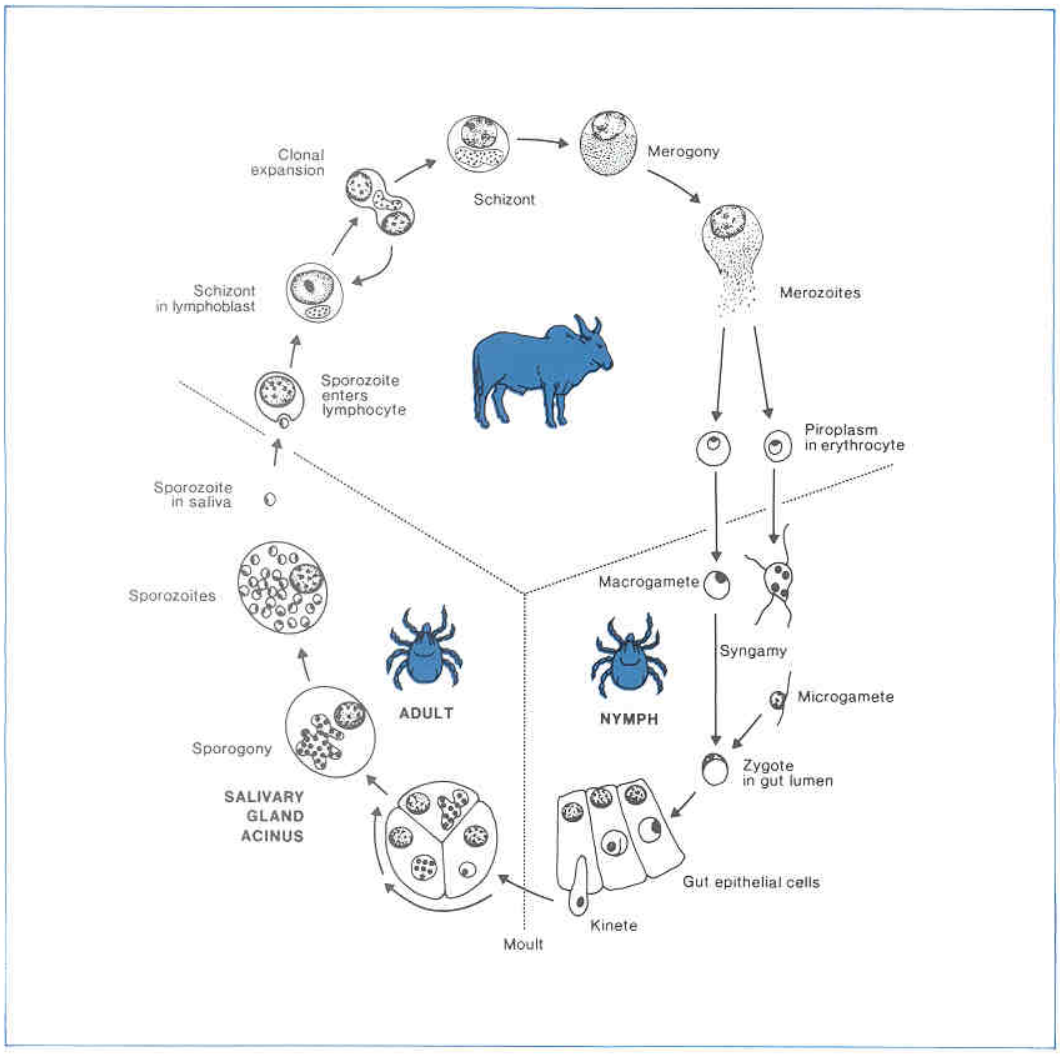

Figure 1.1: The lifecycle of *T. parva*. Image reproduced from and owned by the International Livestock Research Institute.

1.2.3 The tick host

The invertebrate host of *T. parva*, *Rhipicephalus appendiculatus*, commonly known as the brown ear tick due to its preferred feeding site as an adult, is a three host Ixodidae tick. This means that it feeds on three different hosts detaching after each feed and moulting to the next life-stage (larva to nymph to adult) on pasture. *R. appendiculatus* is widespread across much of eastern and southern Africa. Tick populations have an increasingly seasonal pattern the further south from the equator they occur, leading to increasingly seasonal transmission of associated tick borne diseases (Norval et al. 1992). The rate of progression of ticks from one life stage to the next is increased at higher ambient temperatures and their survival increases with increased humidity (Young et al. 1980) . *R. appendiculatus* populations require both a suitable micro-climate created by a mix of grass and tree cover, as well as the supply of suitable hosts to survive (Norval et al. 1992). Therefore tick populations vary locally, and exposures such as overgrazing or an increase in more resistant host species can cause local extinctions (Norval et al. 1992).

R. appendiculatus has a wide host range including many wild ungulates, and carnivores, but cattle are the major domesticated host and are colonised by all life stages. The larvae and nymph stages have less of a preference for feeding near the ears and are found all over the body (Norval et al. 1992). Zebu cattle are relatively resistant to the tick when compared to exotic *Bos taurus* breeds, and become less heavily infected. However, although comparatively resistant, the actual level of resistance developed is low, and has been reported to vary small amounts between individual cattle (Kaiser et al. 1982). However, Walker and Fletcher (1987) observed no differences in the development of resistance to *R. appendiculatus* in experimentally infested calves.

R. appendiculatus are negatively affected by infection with *T. parva* (Watt and Walker 2000). Infected ticks feed more slowly, they produce fewer eggs, fewer of these eggs hatch, and moulting tends to take longer. However, ticks mount a response to their infections (Watt and Walker 2000; Mckeever 2006). Consequently the numbers of sporozoites produced in a tick's salivary acini are much lower than would be predicted from the number of ingested piroplasms. The mechanisms for this are poorly

understood. The level of control or response to infection is believed to vary between ticks (Watt and Walker 2000) and this is one explanation for the over-dispersion of *Theileria* parasites in ticks (Mckeever 2006). Typically around 3% of ticks are infected and often with few infected salivary acini per tick (Young et al. 1986). The massive reduction in parasite numbers from piroplasm to sporoblast is postulated to be a significant population bottle neck for *T. parva* and may exert substantial evolutionary pressure on the parasite (Mckeever 2006; Pelle et al. 2011).

1.2.4 The pathogenesis of and clinical signs associated with *Theileria parva* in the bovine host

The pathogenic effects of an infection with *T. parva* (ECF) are thought to be driven by the invasion, activation, and massive uncontrolled replication of lymphoblasts (both infected and uninfected) (Morrison et al. 1989; Dobbelaere and Heussler 1999; Mckeever 2006) and the severity of the infection is associated with the invasive capacity of those cells (Chaussepied et al. 2010). For a summary of the clinical progression of *T. parva* with approximate timings please refer to figure 1.2. Approximately 5 days following a bite from an infected tick, infected lymphocytes can be detected in the lymph node draining the region closest to the tick bite (Coetzer and Tustin 2004; Norval et al. 1992). Replication of these infected cells in lymph nodes often coincides with pyrexia in the host. Shortly after this, infected cells are detectable in the more distant lymph nodes (Morrison et al. 1989; Coetzer and Tustin 2004). The proliferation of lymphoblasts in the lymph nodes causes the nodes to become hyperplastic and subcutaneous lymph nodes become visibly swollen. The pyrexia is often accompanied by a drop in appetite progressing to anorexia in severe cases. Around this time lymphoblasts are released in large number to the circulation and schizont infected lymphoblasts are sometimes detectable in blood smears. Infected cells can lodge in lymphoid and non-lymphoid parenchymatous organs leading to the development of islands of lymphoid tissue. This is notable and commonly observed on the surface of the kidneys at post-mortem examination as multi-focal white spots, and ante-mortem as clouding of the cornea, especially near the limbus (Coetzer and Tustin 2004). The extensive development of islands of lymphoblastic tissue may disturb

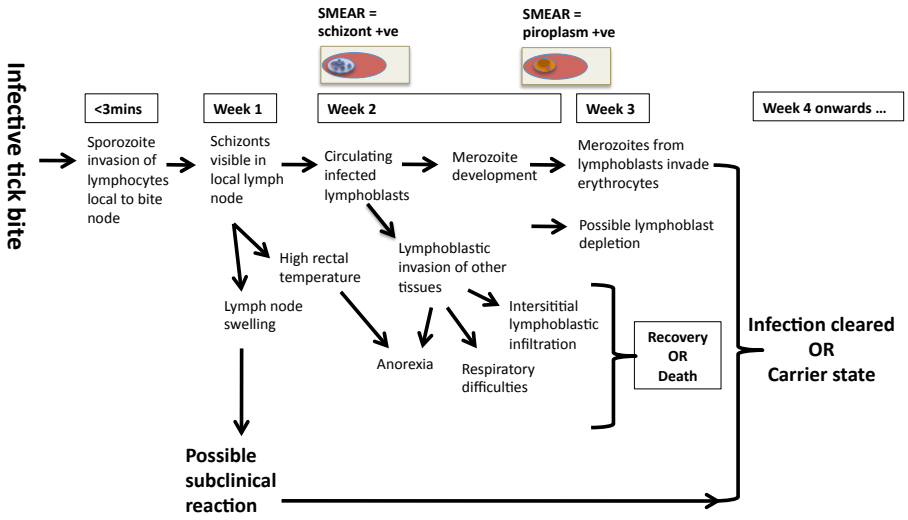

Figure 1.2: Representation of the clinical progression of a *Theileria parva* infection within the cattle host. (Coetzer and Tustin 2004; Norval et al. 1992)

parenchymal organisation and affect organ function. When these islands occur in the gastrointestinal tract the mucosal surface can become damaged and slough off leading to diarrhoea. At post-mortem this phenomenon is often observed as mucosal thickening and ulcers, particularly common in the abomasum. If the liver or kidney's blood supply is disrupted by deposition of lymphoid tissue then areas of necrosis may occur. More rarely, lymphoid tissue may become aggregated in the brain leading to neuropathology and clinical signs such as star gazing and incoordination. These islands of lymphoblasts can be seen on histological examination of affected tissues. This interstitial lymphoblastic infiltration of tissues with frequent mitotic bodies and Koch's bodies should be considered pathognomonic for ECF (Coetzer and Tustin 2004). Respiratory distress (and less commonly froth from the nostrils) is a fulminant sign in terminal cases. This is caused by lymphoblastic infiltration of the pulmonary tissue (Morrison et al. 1989), which leads to activation of the complement cascade and to vasoactive factors being released. This causes disruption in cell junctions and an influx of fluid into the alveoli and bronchial tree. Pulmonary oedema and froth in the bronchial tree is a common and distinguishing post-mortem finding. Disseminated intravascular coagulation is also frequently associated with terminal cases. Death is usually expected to occur 3 to 4 weeks following initial infection (Mckeever 2006; Coetzer and Tustin 2004).

If the immune reaction to the schizont stage of the lifecycle is incomplete and the parasite is not completely cleared from the host the infection continues to progress. A process of lymphoid depletion commences leading to reduced immunocompetence and the potential for secondary infection. This sometimes leads to a more chronic clinical presentation. There is a measureable drop in circulating leukocyte numbers and IgG levels in the blood tend to fall (sometimes detectable as a reduction in total serum protein). The depletion can also lead to a change in the histological presentation of the disease with disrupted cell organisation and cell debris becoming the common finding (Coetzer and Tustin 2004).

Those animals that survive acute disease either recover completely, progress to become an asymptomatic carrier, or may remain chronically affected experiencing poor growth and increased susceptibility to secondary infection. The immune reaction to *T. parva* is strain specific and depending on the profile of infecting strains a host receives they may

not be immune to future infections and may suffer further morbidity or mortality (Taracha et al. 1995).

Although large numbers of erythrocytes become infected with piroplasms, anaemia is not an outcome usually associated with *Theileria parva* (Coetzer and Tustin 2004). Few pathogenic effects are associated with this stage of the life cycle with a non-regenerative anaemia being reported in some cases.

1.2.5 The immune response to *Theileria parva* in the bovine host

High incidence of infection with *T. parva* in endemic areas leads to high levels of antibody in the population, and therefore the number of offspring being born with humoral antibody is high. For many diseases maternally derived antibody provides an immune buffer. The host is exposed to infection early in life and can develop its own adaptive immune response whilst being protected from the pathogenic effects of the infection. In the case of *T. parva*, maternally derived antibodies do not provide such protection (Muhammed et al. 1975) and a cell-mediated response to infection is necessary for immune protection (McKeever et al. 1999; Morrison 2007). *T. parva* replicates within the lymphoblast cytoplasm. It is not within a vacuole, but also, is not exposed to the extra cellular environment at any point during replication (Morrison 2007). Therefore, it is protected from the host's humoral immune response, and antibody is not known to play a role in the immune response to the parasite (Muhammed et al. 1975; McKeever et al. 1999).

As an intracellular parasite, *T. parva* infected cells express highly variable processed peptides on MHC class I molecules (Morrison et al. 1987), which are then presented on the cell surface. In an immune response to *T. parva*, these antigen presenting cells interact with $CD8^+$ T cells to produce a strain specific cytotoxic immune response (Irvin and Mwamachi 1983), which has been shown to solidly protect against homologous challenge (McKeever et al. 1994; Taracha et al. 1995). However, MHC class I types vary between individuals and different MHC types tend to respond to different epitopes of *T. parva* (Irvin 1985; Goddeeris et al. 1990; Mckeever 2006;

Morrison 2007). A host's immune response may concentrate on a single epitope. This leads to a strong immunodominant response, and may explain the strain specificity observed following *T. parva* infection. Therefore, the immune response to *T. parva* results from a combination of the host MHC class I type and the strain of *T. parva*. This variation can cause a problem for the parasite at a population level as it becomes difficult to evolve to escape what are highly individual host responses. This was proposed to maintain the extensive local variation in parasite strain (Morrison 2007; Pelle et al. 2011). However, the selection in the tick where sexual recombination occurs must be assumed to be a major source of selection.

T. parva is more virulent in exotic breeds, than in indigenous breeds such as the short horn zebu. The mechanisms behind the apparent tolerance of short horn zebu cattle to ECF is poorly understood. However, a similar tolerance is observed in infections of *T. annulata* in the indigenous Sahiwal breed of cattle when compared to the response of exotic breed Holstein-Friesians. *T. annulata* has many similarities to *T. parva*, but a different geographical range. The relationship between *T. annulata* and both Sahiwal and Holstein-Friesian cattle has been investigated in some detail. Holstein-Friesian cattle suffer more disease associated with *T. annulata* when compared to local indigenous breeds (Glass et al. 2005). The Sahiwal have been observed to have a much reduced activation of macrophages, the main mechanism of pathogenesis for *T. annulata* (equivalent to *T. parva* activation of lymphocytes) when compared to Holstein-Friesian cattle (Glass and Jensen 2007). This has been shown to be due to the increase in the production of TGF-β2 induced by the parasite in Holstein-Friesian compared to Sahiwal cattle, which increases the invasive potential of *T. annulata* infected macrophages (Chaussepied et al. 2010). Although a different cell type is responsible for the pathogenesis, it is possible that a similar mechanism of tolerance occurs in short horn zebu cattle when infected with *T. parva*.

1.2.6 Current understanding of host variation in response to *Theileria parva*

Clinical outcomes following *T. parva* infection are influenced by the infective dose of the parasite, the breed of the cow, and individual variation of both the parasite and host

(McKeever et al. 1999). Mechanisms behind differences in host outcomes have been the topic of experimental studies. These have investigated both pathogen and host effects. Infective dose was reported to affect both severity of disease and time to onset of clinical signs (Radley et al. 1974; Morrison et al. 1996). Also, different strains of parasite were observed to have different pathogencity (Radley et al. 1974; Irvin et al. 1989; Tindih et al. 2010). Finally, variation in the adaptive immune response mounted by cattle following infection has been investigated (Morrison et al. 1996; Dobbelaere and Heussler 1999). In a hypothetical situation where several animals are infected with the same dose of parasite, the cell mediated $CD8^+$ T cell response has been shown to vary by individual. However, there are several other factors that are likely to influence that host response. This includes the cell type initially infected (if sporozoites infect T lymphocytes a more severe clinical reaction is observed compared to if B lymphocytes are infected (Morrison et al. 1996)). Also environmental exposures including maternal effects, previous infection history, and co-infections are likely to affect host outcome. The heterogeniety of clinical outcomes following infection with *T. parva* have been recorded in observational studies in endemic regions (Moll et al. 1984, 1986; Gitau et al. 1999; Maloo et al. 2001; Gachohi et al. 2010). Differences in host response have also been demonstrated following homologous challenge during experimental studies (Irvin et al. 1989). The risk factors that have been found to be associated with increased morbidity and mortality are the husbandry system used (herded grazing as opposed to stall fed) (Gachohi et al. 2010; Gitau et al. 1999; Maloo et al. 2001), the number of animals kept (Gachohi et al. 2010), and the agroecological zone (which is often associated with elevation and is a proxy for vegetation cover and climate) (Gitau et al. 1999; Maloo et al. 2001). These risk factors, although associated with host outcome were possibly correlated with exposure rather than pathogenicity following infection. The burden of disease of all sorts is highly influenced by the environment (Smith et al. 1999). Overcrowding, poor sanitation and water quality predispose to increased transmission of certain potential pathogens, which in turn can lead to further contamination of the environment. Environmental stress and poor nutrition may decrease the effectiveness of the immune system (Chandra 1997), and are likely to lead to poorer outcomes following infection. As these risk factors are not pathogen specific, they are likely to lead to increased frequency of clinical disease of all causes.

Mothers affect the way their offspring respond to infection, through their genotype and the way this interacts with environmental exposures (Marshall and Uller 2007). For example, the maternally conferred protection from mother to offspring in colostrum will depend on the quality and quantity of the colostrum (genotype and nutritional status) and the infections to which the mother was exposed and her immune responses (genotype, health status, and infection pressure).

Individuals will be more or less suited to the environmental conditions they experience. This will affect their outcome following infection, and may cause ill health to cluster within certain individuals.

1.2.7 The diagnosis of *Theileria parva*

Clinical diagnosis

Clinical diagnosis of *T. parva* is often made in the field based on a combination of clinical signs and identification of the tick vector. Aspirates from hyperplastic lymph nodes in clinical cases will often yield schizont infected lymphoblasts. Smears can be made from aspirates and cells are stained with Giemsa to visualise the parasites. Semi-quantitative counts of the parasite can be made through examination of the smears under a light microscope (Norval et al. 1992). Central or peripheral blood smears are also useful yielding both schizonts in circulating lymphoblasts and piroplasms in erythrocytes. Blood and lymph node smears are easy to make and stain and although reliant on electricity to run a high quality microscope and on the availability of an experienced technician, they offer an accessible diagnostic method in endemic regions. However, the piroplasms and schizonts of many of the *Theileria* species look similar or even identical making identification to species level incredibly difficult if not impossible by microscopy. In fatal ECF cases, post-mortem examination offers the opportunity to observe gross pulmonary changes, tissue damage, and islands of immune tissue in parenchymal organs. Observation of interstitial lymphoblastic infiltration of parenchymal organs with the presence of Koch's bodies on histological examination of tissue sections, is characteristic and pathognomonic for ECF (Coetzer and Tustin 2004).

Serological testing

ELISAs (Enzyme Linked ImmunoSorbant Assays) have been developed for the identification of antibodies raised to *T. parva* and *T. mutans* (Katende et al. 1998, 1990). Indirect antibody ELISAs rely on a colour change to detect the presence of antibody in a sample. A specific antigen bound to a plate binds the antibody present in a sample. This is in turn bound by an antibody to the host antibody, in this case an antibody to bovine IgG (anti-bovine immunoglobulins). The anti-bovine antibody is bound to an enzyme to form a conjugate. This conjugate binds proportionately to the amount of antibody of interest bound to the plate. Substrate is added which is modified by the enzyme present to cause a colour change (Crowther 2001). This colour change is measured by a spectrophotomter. A *T. parva* specific indirect ELISA protocol published by Katende et al. (1998) utilises IgG antibody raised to the schizont polymorphic immunodominant molecule (PIM) molecule.

As for all antibody based ELISAs this test identifies exposed, but not necessarily currently infected, individuals. Interpretation of results from ELISA testing are also complicated in young animals due to the presence of maternally derived antibody which may persist for some months. Moll et al. (1984) observed antibodies to *T. parva* and *T. mutans* to persist for around 3.5 months.

Polymerase chain reaction based tests

Polymerase chain reaction (PCR) assays have been developed for *T. parva* detection. This can either be as a single species PCR which has most recently applied the amplification of the p104 region of the genome (Skilton et al. 2002; Odongo et al. 2010), or as part of a multiple species assay (the Reverse Line Blot (RLB)), which simultaneously tests for all the common tick borne diseases in the region (Gubbels et al. 1999; Bazarusanga et al. 2007; Odongo et al. 2010).

The p104 based PCR for *T. parva* has most recently been developed as a nested PCR which can be applied to blood samples collected from peripheral ear veins and applied to FTA cards (Odongo et al. 2010). Blood spot samples are very easily collected and FTA cards can be stored at ambient temperature and very easily and cheaply

transported.

A reverse line blot is a polymerase chain reaction (PCR) based test that uses generic primers to amplify regions of target species genomes. In this case, the 18S small sub-unit rRNA gene (Gubbels et al. 1999). Probes were designed to hybridise to species specific oligonucleotides in the hyper-variable V4 region of the 18S gene. These species specific probes are immobilised on a membrane. The probes are loaded perpendicular to the PCR products and hybridisation occurs at specific points on the membrane for specific parasite species. Hybridisation is then visualised by binding and activation of a chemiluminescent substance which exposes an x-ray film. RLB allows for several samples to be tested for several species simultaneously.

1.2.8 *Theileria parva* at a population level

Theileria parva is endemic in the extensively grazed indigenous cattle population of Kenya. In several regions, including Western Kenya, the infection has been shown to be present at high levels in the cattle population and in all ages (Barnett 1957; Moll et al. 1984; Latib et al. 1995; Maloo et al. 2001; Okuthe and Buyu 2006; Gitau et al. 1997), a state referred to as hyperendemic (Thrusfield 2005). Estimates of seroprevalence varied between 62.8% (Moll et al. 1984) and 79% (Maloo et al. 2001).

Where indigenous animals with a low innate susceptibility to disease are herded extensively, where *R. appendiculatus* is common and the population is stable over the year, and where the proportion of infected ticks is low then a state of endemic stability is expected (Norval et al. 1992). The prevalence of exposed individuals is high and clinical disease cases are few.

Endemic instability, where clinical disease is common and the prevalence of exposure low, is expected where animals have a high innate susceptibility to disease, or where exposure is variable over the year due to seasonal variation in the vector population, or restricted exposure is imposed by zero-grazing management or intensive acaricide treatments (Norval et al. 1992).

It has been proposed that endemic stability requires 2 criteria. These are that "disease, at least over some age range, is a more likely outcome of infection ... in older than

younger animals", and that "initial infection decreases the probability ... that subsequent infections result in disease" (Coleman et al. 2001). However, for *T. parva* neither of these criteria are met. Maternal antibodies offer no protection against disease, and there is no evidence for innate resistance in young animals as for babesiosis, for example (Coetzer and Tustin 2004), and the strain specific immune response characteristic of the bovine response to *T. parva* does not necessarily protect against heterologous challenge.

A mechanism for endemic stability of *T. parva* has been proposed. The carrier state is established in animals that control their initial *T. parva* infection. Following this initial infection a few infected cells remain in lymphoblasts within lymphoid tissue (Skilton et al. 2002; Morrison 2007). These continue to divide at a low rate, leading to the very low number of piroplasm infected circulating erthrocytes. A low rate of infection in ticks is thought to be maintained by carrier animals from which the tick consumes only very few infected erythrocytes (Norval et al. 1992). Therefore, when calves are first infested with *R. appendiculatus*, even when several ticks are feeding, the number of sporozoites to which the calf is exposed are few, and this is postulated to reduce the risk of overt clinical disease following initial exposure (Radley et al. 1974). Repeated low exposure to different strains would then be hypothesised to lead to a gradual development of solid immunity.

1.2.9 The treatment, management, and prevention of *Theileria parva*

There are three drugs available for the treatment of ECF. Oxytetracycline treatment is effective if given early in the course of disease. However, a significant fall in white blood cell count can still occur in treated individuals (Dolan et al. 1984). Buparvaquone, an anti-protozoal compound, is recommended to be given in two doses, and has been shown lead to a good recovery rate. However, animals can remain carriers and may experience a low grade chronic ECF for some months leading to a loss in productivity (Dolan et al. 1992). Halofuginone, a quinazoline, is effective, but has a very narrow therapeutic index and can cause severe diarrhoea.

The anti-parasitic effects of oxytetracycline are also used prophylactically as part of the infect and treat vaccination method (ITM). The strain specific cell mediated immune response to *T. parva* has made the development of an effective and safe vaccine challenging. The ITM vaccine involves the inoculation of live sporozoites to calves, with a simultaneous injection of long acting oxytetracycline to reduce any clinical disease in vaccinated animals. The sporozoites are presented in material made from ground up ticks. This has to remain frozen until inoculation. The inoculate is made up of a mix of strains known as the Mugaga cocktail, which includes stabilates known as Muguga, Serengeti-transformed, and Kiambu 5. The genetic diversity of strains in this cocktail have recently been characterised using 5 satellite markers (Patel et al. 2011), which demonstrated broad heterogeneity with some overlap of strains between the stabilates. This mix of strains appears to offer solid protection against ECF mortality. The method is cost effective for controlling ECF, particularly in pastoralist systems (Martins et al. 2010) where calf losses are generally higher (30-60% without prevention). It also reduces the need for acaricide use, reducing both costs and the development of resistance (Kivaria et al. 2007). However, its use poses several problems that have prevented its widespread use. Batches can be variable and need careful formulation, the production process is involved and requires passage of the parasite through both rabbits and cattle, and stabilates need to be stored in liquid nitrogen following preparation up until inoculation. There has been a concern about the widespread use of oxytetracycline as part of a prophylactic protocol, and because a carrier strain is induced in inoculated animals there is a possibility that strains in the vaccine can be introduced to areas where these strains were not previously found (McKeever et al. 1999; Di Giulio et al. 2009). This was demonstrated to have happened following use of ITM in Zambia, Kenya, and Uganda (Geysen et al. 1999). The implications, if any, of such introductions are yet to be described, but because the variation in naturally occurring populations is high anyway, and carrier animals are often transported to new regions with their strains, the size of the impact has been predicted to be minimal (Di Giulio et al. 2009).

The control of ECF is possible through intensive acaricide treatment, and was achieved in some regions of South Africa where tick populations vary with season and contact with wildlife was able to be controlled (Norval et al. 1992). However, up to twice

weekly acaricide treatment is required to keep tick numbers low enough to affect a substantial reduction in disease risk in exotic cattle herds. Acaricides can be harmful to the environment and to those applying the chemicals, and over time resistance tends to develop so reducing effectiveness of the treatments (Homewood et al. 2006). Intensive use of acaricide upsets an endemic disease state when present, and when indigenous cattle are present any breakdown in tick management is likely to lead to more rather than fewer losses due to disease.

Another common method of control, not requiring the use of pharmaceutical products, is zero-grazing. This is popular with those small holder dairy farmers keeping exotic cattle or their crosses. If cattle are restricted to stalls and fodder is brought to them then their risk of exposure to ticks and so to *T. parva* is much reduced. However, it is not reduced to zero as ticks may be brought in with cut fodder and so disease still sometimes occurs. A state of endemic instability is set up when using such practices, and when infection is introduced losses can be substantial.

Most commonly, however, no control is practised at all. All the time that conditions favour an endemically stable state, those farmers who extensively graze indigenous cattle hope to only rarely encounter *T. parva* associated clinical disease and mortality.

1.3 *Other important infections of the region, and the concept of co-infection*

1.3.1 Other species of *Theileria*

T. mutans, *T. taurotragi*, *T. velifera*, and *T. sable* are other *Theileria* species that would be expected to co-circulate in the east African domestic cattle population (Norval et al. 1992; Coetzer and Tustin 2004; Bazarusanga et al. 2007; Chaisi et al. 2013). There have been several reports of clinical disease associated with *T. mutans* (Bazarusanga et al. 2007; Robson et al. 1977; Young et al. 1978; Saidu et al. 1984). However, it is widely believed to be non-pathogenic and continues to be considered as having no clinical importance beyond confusing the diagnosis of *T. parva* in blood smears (Chaisi

et al. 2013). The parasite was observed to cause a drop in packed cell volume in splenectomised calves, but with no other reported clinical signs (Brocklesby et al. 1972). However, co-infection of *T. mutans* with *T. taurotragi* was reported to cause morbidity in Botswana in dairy breeds (Binta et al. 1998). *T. mutans* is transmitted in the study region by the tick, *Amblyomma variegatum*, which coexists with *R. appendiculatus*. Similar to *T. parva*, *T. mutans* is taken up by lymphocytes in the cattle host, but unlike *T. parva* the main replication of the parasite in the bovine host occurs in the erythrocytes. Therefore, the piroplasm is the dominant stage of the lifecycle, with the schizont stage being present but somewhat fleeting. Very little research has been carried out into this parasite and there is little understanding of either its epidemiology or its effects on the host. *T. taurotragi* is also believed to be mostly benign in cattle causing a pyrexia with mild lymph node enlargement in some cases. The cerebral manifestation of *T. taurotragi*, common in eland, rarely occurs in the bovine host (DeVos 1982). Like *T. parva*, *T. taurotragi* is transmitted by *Rhipicephalus appendiculatus* (Young et al. 1977). *T. velifera* and *T. sable* are also believed to be non-pathogenic organisms and again only of importance as they may be confused with *T. parva* in diagnostic blood smears (Coetzer and Tustin 2004). All these species attract little research and there is only limited information available about their biology or epidemiology.

1.3.2 Other vector borne diseases

Babesisosis

Babesiosis (the most common cause of which in this region is considered to be *Babesia bigemina* although *Babesia bovis* may occur) leads to extensive intravascular haemolysis, severe anaemia and the classic sign of red urine caused by excreted haemoglobin released from lysed erythrocytes (the disease is also referred to as red-water) (Coetzer and Tustin 2004)

Anaplasmosis

Anaplasmosis (*Anaplasma marginale*) is often subclinical in those calves under one year old with increasing severity with age for those individuals not infected during early life. The common pathology is anaemia which can be severe, sometimes critically. Pyrexia is frequently seen. Icterus follows the acute stage and usually suggests that the animal will recover but this is a long process with extended convalescence (Coetzer and Tustin 2004)

Heartwater

Heartwater (caused by *Ehrlichia ruminantium* infection) leads to pyrexia of three to six days duration. Diarrhoea is a reported sign. Nervous disorders including hypersensitivity occur late in disease progression possibly leading to convulsions. Severe hydropericardium is the common indicative sign seen at post-mortem examination (giving the disease the name 'heartwater'). Pulmonary oedema is also a common sign and leads to froth in the bronchial tree. Lymph node hyperplasia, abomasal fold swelling, and nephritis are other common findings (Coetzer and Tustin 2004).

Trypanosomiasis

Trypanosomiasis is endemic in cattle in Kenya where its vector, the tsetse fly, occurs. The clinical signs associated with the disease are chronic anaemia and wasting, a rough staring coat, weakness and depression (Maudlin et al. 2004). The species of *Trypanosoma* circulating in cattle populations in Western Kenya, are *T. vivax*, *T. congolense*, and *T. brucei* brucei (Thumbi et al. 2010; Bronsvoort et al. 2010). *T. vivax* and *T. congolense* are the more pathogenic of the three for cattle.

1.3.3 Parasitic gastroenteritis

The gastrointestinal worm, *Haemonchus contortus* is very common (Latib et al. 1995) and heavy infestations of the abomasum by this blood sucking worm lead to severe anaemia (which can become non-regenerative following extended periods of iron losing blood loss). Damage to the abomasal mucosa by the worm disrupts digestion and can lead to reduced nutrient absorption, and a protein losing enteropathy. In severe infestations the combination of poor nutrition and severe anaemia can result in death (Urquhart et al. 1996).

Other species of endoparasite common in this region include *Trichostrongylus axei*, *Oesophagostumum radiatum*, *Cooperia* species (Waruiru et al. 2001), *Nematodirus*, *Strongyloides* (Waruiru et al. 1998), *Toxocara* species, and the lung worm, *Dictyocaulus vivparous*. It is usual for individuals to become infected with gastrointestinal parasites from a very young age from soon as they begin grazing, but the infections often remain subclinical. However, high levels of infection lead to damage to the intestinal mucosa, and a protein losing enteropathy leading to weight loss and possibly death in very heavy infestations (Urquhart et al. 1996).

1.3.4 Co-infection and host outcome

It is the norm for individuals to be infected with several different parasite species at any one time, and these infections can, in some cases, interact in synergistic or antagonistic ways (Cox 2001; Pedersen and Fenton 2007). Interactions between these co-infections can be via competition for resources, or modification of the host's immune response, and the effects can be of benefit or detriment to both the parasite or the host.

Previous studies in wild populations have often concentrated on the effects of co-infection on parasite populations within a host (Lello et al. 2004; Telfer et al. 2010). Those in humans have often investigated the effects on the host, for example HIV on hepatitis C (Greub et al. 2000) or parasitic worms on malaria (reviewed in Nacher (2011)). Such work investigating interactions between species of parasites colonising free living populations needs intensive longitudinal study, which requires a large investment of both money and time. Most literature on the topic of co-infection report

a positive effect on parasite abundance, or a negative effect on host outcome (Griffiths et al. 2011).

For those infections that reduce the function of the immune system, the effect on the host of co-infections with potential pathogens is predictable. HIV, in its suppression of the immune system, enables many other potential pathogens, that may otherwise have remained quiescent, to establish in a host and cause serious clinical disease. However, in the case of malaria and gastrointestinal worms, the interactions are complicated and less predictable (Nacher 2011). *Ascaris* worms are believed to protect the host against clinical malaria. However, hookworms tends to increase the incidence of the disease. This poses problems for integrated parasite control policies using broad spectrum anthelminthics and is a good illustration of the complex nature of within host parasite interactions. It has recently been shown that co-infections of *Fasciola hepatica* (fluke) with *Mycobacterium bovis* (bovine tuberculosis, bTB) in cattle can reduce the levels of interferon (IFN) γ produced by peripheral blood monocytes (Flynn et al. 2009). Although experiments did not show a change in the course of either disease when in co-infection, the fluke was shown to modify the immune response compared to when bTB alone infected calves. Although the clinical impact of this co-infection is poorly understood, this interaction does have the potential to affect the results of the IFNγ test used to identify cases of bTB, and so an impact on the ability to control a notifiable disease. The effects of co-infections can be wide ranging and unexpected.

1.4 Summary of thesis

Western Kenya is an area in which animals are assaulted by a barrage of potential pathogens from birth. The aim of this thesis is to investigate why, in this high infection pressure environment, some calves faltered while others thrived. The focus of the thesis is *Theileria parva*, believed to endemically stable in the study region. Risk factors for infection are sought, the clinical outcomes in calves are described, and risk factors for ill health are investigated. Finally, risk factors for mortality following infection with *T. parva* are described.

Chapter 2 describes the IDEAL study, and methods used throughout this thesis.

Chapter 3 describes *Theileria parva* in the study region and its relationship to other species of *Theileria*, and investigates risk factors associated with seroconversion to both *T. parva* and *T. mutans*. Chapter 4 describes the clinical signs associated with clinical episodes and death, it describes the variation in clinical presentation between calves with a focus on ECF, and explores the use of a clinical decision support tool for diagnosing ECF in the field. Chapter 5 describes the frequency, and characteristics of clinical episodes in the calf cohort, and investigates risk factors associated with being a sick calf. Chapter 6 investigates risk factors for ECF mortality following infection with *Theileria parva*, using a nested unmatched and matched case control format. Finally, chapter 7 discusses the findings of this thesis.

Chapter 2

The IDEAL study design and data analysis

2.1 Background

The study design and visit protocol along with summary findings from the IDEAL project were published in BMC Veterinary Research (Bronsvoort et al. 2013). This paper is held in appendix A. Those parts of the study design and methods relevant to this thesis are described below.

This project was approved by the University of Edinburgh Ethics Committee, the Kenyan Department of Veterinary Services and by ILRI's Animal Care and Use Committee. Standard non invasive techniques were used to collect blood and faecal samples for diagnosis and identification of disease and infection. The calves were restrained by professional animal health assistants and veterinary surgeons and a veterinary surgeon was available to examine any sick calf reported by recruited farmers. Farmers were contracted to contact the project to request a visit when calves were ill or injured and any calves which were in severe distress due to trauma or disease were humanely euthanised by a veterinary surgeon. All farmers gave informed consent in their own language before recruitment of their calves began. The Ethical Review Committee of the University of Edinburgh (Animal (Scientific Procedures)

Act, 1986) took into account the ethical issues enshrined in the Animals (Scientific Procedures) Act and approved the work (reference number OS 03-06).

The study area was located in Western Kenya, and was a 45Km semi-circle radiating south-east from Busia town on the Ugandan border. The study area covered 5 different agroecological zones (AEZs). Agroecological zone classification describes the type of land and its suitability for different crops and combines data on soil, topography, and climate (FAO 1996). AEZ was used to stratify the random sampling. The longitudinal design of IDEAL required calves to be visited regularly. To make the visits possible it was necessary to recruit calves in geographical clusters. These clusters were within sublocations (SL). These are the smallest administrative unit in Kenyan governmental organisation and are typically around 10 Km across containing 80 to 90 households. A list of all sublocations within the study area was compiled, those sublocations representing urban centres were excluded, and the sublocations were then stratified by AEZ. Sublocations were randomly selected from each AEZ proportional to the number of sublocations in that AEZ. A total of 20 sublocations were selected across five AEZs. To clarify, this was a two stage random stratified clustering design with stratification on AEZ. Figure 2.2 is a map of the study site showing the AEZs and randomly selected sublocations (map reproduced with permission from Samuel Thumbi). Around two calves were recruited from each sublocation every five weeks. This allowed the birth dates of calves recruited within all sublocations to be equally distributed across the entire recruitment period. Over the two year recruitment period, in total between 22 and 28 calves were recruited to the study per sublocation giving a total of 548 calves. The number of calves recruited by sublocation is summarised in table 2.1. Figure 2.1 summarises the study design and visit protocol.

2.2 The Visit Protocol

2.2.1 Recruitment criteria

The study aimed to investigate the infections and outcomes of indigenous short horn zebu calves. To avoid recruitment of exotic breeds and cattle recently brought in from

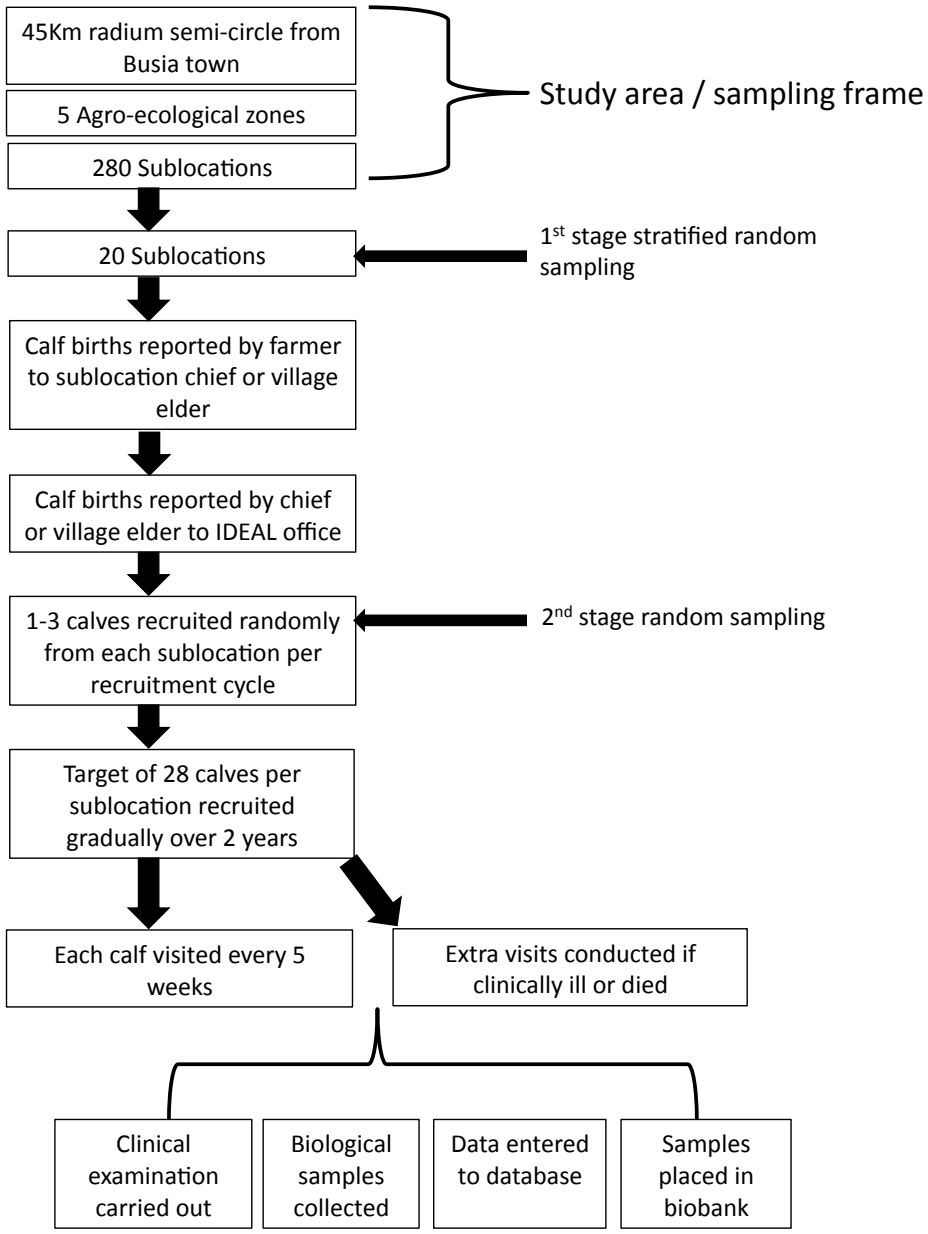

Figure 2.1: Flow diagram summarising the study design and visit protocol

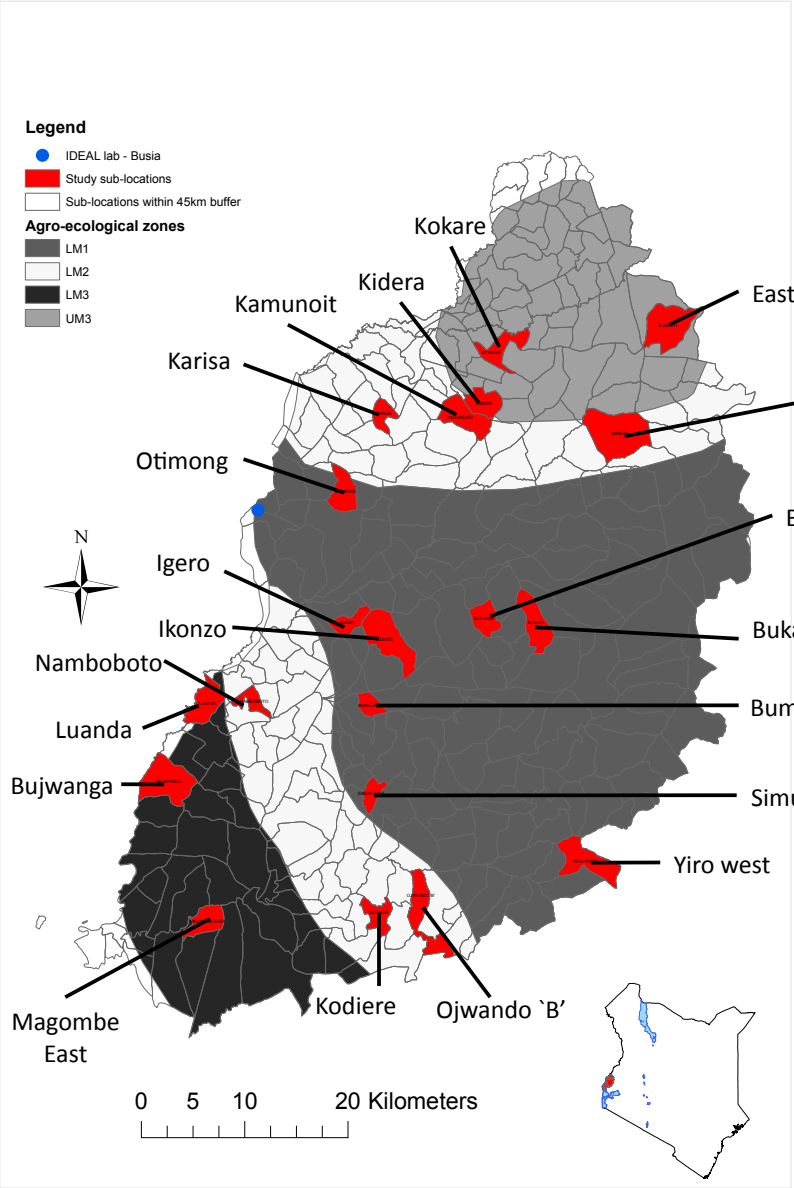

Figure 2.2: Map of the study site. Permission for reproduction kindly granted by Samuel Thumbi

Table 2.1: Summary of information on the 20 randomly selected sublocations including the number of recruitments made. SL ID = identification number for sublocation. AEZ ID = identification number for agroecological zone.

Sublocation	SL ID	No.calves	AEZ ID	AEZ description
East Siboti	1	28	01	UM3
Kidera	2	28	01	UM3
Kokare	3	28	01	UM3
Mabusi	4	28	02	LM2 middle
Kamunuoit	5	27	02	LM2 middle
Karisa	6	28	02	LM2 middle
Otimong	7	28	03	LM1
Igero	8	28	03	LM1
Bulwani	9	28	03	LM1
Bukati	10	28	03	LM1
Ikonzo	11	28	03	LM1
Bumala A	12	22	03	LM1
Yiro West	13	28	03	LM1
Simur East	14	27	03	LM1
Namboboto	15	27	04	LM2 south
Ojwando B	16	28	04	LM2 south
Kodiere	17	27	04	LM2 south
Luanda	18	28	05	LM3
Bujwanga	19	28	05	LM3
Magombe East	20	26	05	LM3

outside the study area it was a requirement that; 1) calves had to have been born within three and seven days of the date of recruitment, (2) they had to be born to a dam that had been on the farm for at least one year, (3) they must not have been born from artificial insemination, (4) the herd had to be extensively grazed, and (5) only one calf from a farm could be a member of the study at any one time. The final stipulation was to prevent over-representation of large herds (and therefore richer households) in the study.

2.2.2 Recruitment protocol

Calf births were reported to the IDEAL office by the sublocation chief or village elder. The recruitment team visited every sublocation every five weeks and attempted to recruit the required number of calves (usually one to three). Calves were randomly

selected by pulling a name from a hat. Each homestead was visited, the project was described in the language of the head of the household and informed consent was gained. If consent was denied the reasons for this were recorded. The farmers were asked to agree to 5-weekly routine visits from an IDEAL team and to weekly visits by an IDEAL trained local animal health assistant (LAHA). They were requested to manage the calf as they would any calf in their herd, but to not treat the calf with any drugs at any point during its membership of the cohort. It was requested that any incidents of ill health and or calf death were reported immediately to the village elder or directly to the IDEAL office. It was also requested that the IDEAL teams had access to the calves' dams. Recruited calves were bought by the project at the current market rate and were returned to the farmers care and ownership at the end of the study. Farmers were compensated for calves that died with a one year old locally bought calf.

On successful recruitment to the study both the calf and the dam were ear tagged in both ears with bar coded tags. Following this, an extensive questionnaire was carried out (appendix C). This collected information about the calf owner, the homestead (the farm), the cattle herd and other animals kept on the farm, and the typical cattle management practices in the homestead. The number and species of animals kept on each farm was converted to tropical livestock units (TLU). This is a method that uses the mean weight of different species to calculate a compound value representing the total livestock kept. One TLU is defined as 250Kg, equivalent to 1 cow, 10 goats or sheep, 5 pigs, 100 chicken, and 0.7 camels (ILCA/ILRAD 1988). The dam was examined and information on its state of health and measurements were collected (questionnaire is in appendix D). The dam's heart girth was measured, its udder and teats were examined, and the milk was checked from all four quarters for clots.

2.2.3 Routine visit protocol

Once recruited the calves were visited approximately every 5 weeks and a routine protocol was carried out. The routine visit protocol is summarised in figure 2.3. The questionnaire associated with this protocol is in appendix E.

The routine visit followed the format of a veterinary consultation but findings were

recorded using tick boxes and codes to reduce inter-operator variability. The farmer was asked about the health of the herd and that of the individual calf. The calf was then observed at rest and any abnormalities noted. The attending animal health assistant or vet then carried out an extensive clinical examination. The calf's rectal temperature was taken. Eyes, ears, nose, umbilical, and urogenital openings were checked for discharges. The peripheral lymph nodes were palpated and their width measured using calipers. Mucous membranes were assessed for pallor and were also assessed using FAMACHA (Reynecke et al. 2011), a card with tones of red that can be used to score anaemia. Then the whole body surface was palpated, abnormalities noted, and any adult ticks recorded by species and as either present or absent. Species identification was carried out by the attending animal health assistant or vet following training from the senior vet on the project. Nymph and larvae stage ticks were not recorded, and a tick count was not carried out. The girth of calves was measured using a measuring tape placed around the thorax just caudal to the scapula. Tension was maintained constant on the measuring tape by applying pressure to one end of the tape using a spring balance pulled to 1 Kg. The weight of the calves was measured at visits at weeks one to 31, and at week 51. The weight at the early visits was taken using a sling and a spring balance. The weight at the final visit at 51 weeks was taken using digital weigh beams and a weigh bridge. Finally, blood smears, whole blood, serum, and faeces were collected. At the end of the visit it was decided whether the calf was experiencing a clinical episode (see later, section 2.2.4). When calves were clinically ill, clinical samples were collected in addition to routine samples to help with diagnosis of the case.

Faecal samples were split. One was transported at ambient temperature and one was transported in a cool box with ice packs. Blood samples were also transported in cool boxes. Smears were made in the field, air dried, and were transported back in slide boxes at ambient temperature. All samples were labelled on the farm with barcoded labels and linked to the calf or dam identity numbers at the time of sampling using a bar code reader. Samples collected from calves were transported back to the field laboratory daily. They were logged in the database and a laboratory work sheet was automatically generated that listed all routine testing to be carried out. Samples were refrigerated over night (apart from blood smears and one half of the faecal samples

which were kept at room temperature). All samples were processed and tested the following day.

2.2.4 Clinical episode and post-mortem visit protocol

Clinical visits outside of routine visits were triggered following a visit by a local representative of the IDEAL project or by the farmer. These local representatives were usually animal health operatives (often agrovet workers or local veterinary office employees. They were referred to as local animal health assistants (LAHA). They received further training from the IDEAL project veterinarians to enable then to carry out a basic clinical examination and collect clinical data according to IDEAL protocols. There were asked to take a rectal temperature, and were asked to assess lymph node size, and the calf's general demeanour. They made weekly visits to every calf still enrolled in the project within their local area. If any of the calves were found to be sick the project office was contacted. The IDEAL project veterinarian organised a visit to the homestead and the calf received an extra visit where it was clinically assessed and samples taken. The farmer themselves could also trigger an extra visit by directly contacting the IDEAL project office about ill health in, or death of, their calf. At each of these visits and also at all routine visits the attending AHA or veterinarian defined the calf as suffering from a clinical episode or not. For consistency, a definition of a clinical episode was established. The decision was made according to a protocol defining the signs and combinations of signs that should trigger the assignment of a clinical episode. A card was used in the field as an aide memoire and this can be seen along with the accompanying notes in figures 2.4 and 2.5. This decision was made following completion of a detailed physical examination of the calf. This included measurement of rectal temperature, assessment of mucous membrane pallor, measurement of the peripheral lymph nodes, and an assessment of general demeanour (e.g lethargy, nervous signs). The farmer was asked to report on changes in appetite or thirst. Although the definition was necessarily prescriptive, there was also the opportunity for the operator to make personal judgements when considering combinations of signs. The veterinarian on duty was consulted when there was uncertainty about a case. All post-mortem visits were categorised as clinical episode

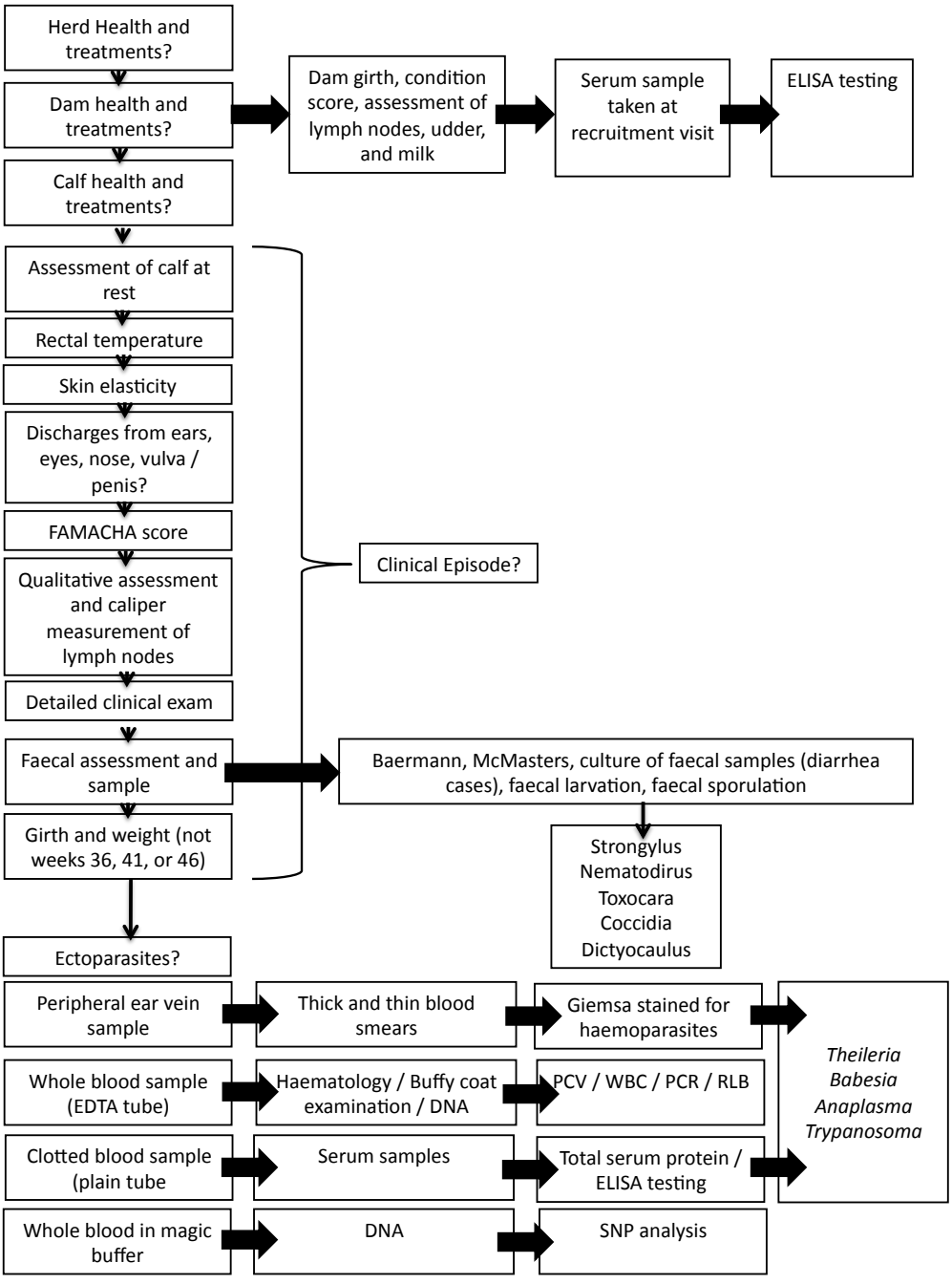

Figure 2.3: Flow diagram summarising the study design and visit protocol

visits. If the calf was deemed to be suffering this suffering was alleviated either through treatment or by euthanasia. If a calf was treated it was dropped from the study from that visit, and follow up was carried out to ensure the welfare of the calf. Calves that were euthanised received a full post-mortem examination and the farmers were compensated as described previously.

2.2.5 Post-mortem examination

Calf owners were requested to report the death of study calves to the project office. Following alert, the homestead would be visited as soon as possible. On arrival the person responsible for the care of the calf was interviewed about its clinical history leading up to death and about any ill health in the rest of the herd. The post-mortem examination was carried out either at the homestead and the carcass buried, or the calf was transported back to the field laboratory in Busia and the post-mortem conducted there. Recording of mortality was complete, but not all calves received a post-mortem visit.

Prior to opening the carcass a peripheral blood smear was made (usually from blood extruded from the ear), stained with polychrome methylene blue, and examined for anthrax. If confirmed negative the post-mortem continued (no positive smears were found during data collection). Carcasses had a full external examination and were then skinned from the midline, the pluck removed and fully examined, the abdominal organs systematically sliced and examined and the intestines entirely opened for inspection of both the serosal and mucosal surfaces. Major articulated joints were opened. The skull was split and the cerebral cortex, meninges and brain were examined. A smear of cerebellum was made, dried, stained, and examined for *Ehrlichia ruminatum*. *Ehrlichia ruminatum* can often be observed in the capillaries of the brain in clinical cases (Coetzer and Tustin 2004). All observed abnormalities were recorded. Routine tissue samples were taken in replicate for freezing at -80^{0}C and also into 10% formalin for histological examination (lungs, heart, kidney, spleen, liver). Impression smears were made from peripheral lymph nodes and spleen, were air dried, fixed, and stained using Giemsa. Any extra samples deemed necessary for diagnoses were collected and processed appropriately according to the sample type. All waste material from the

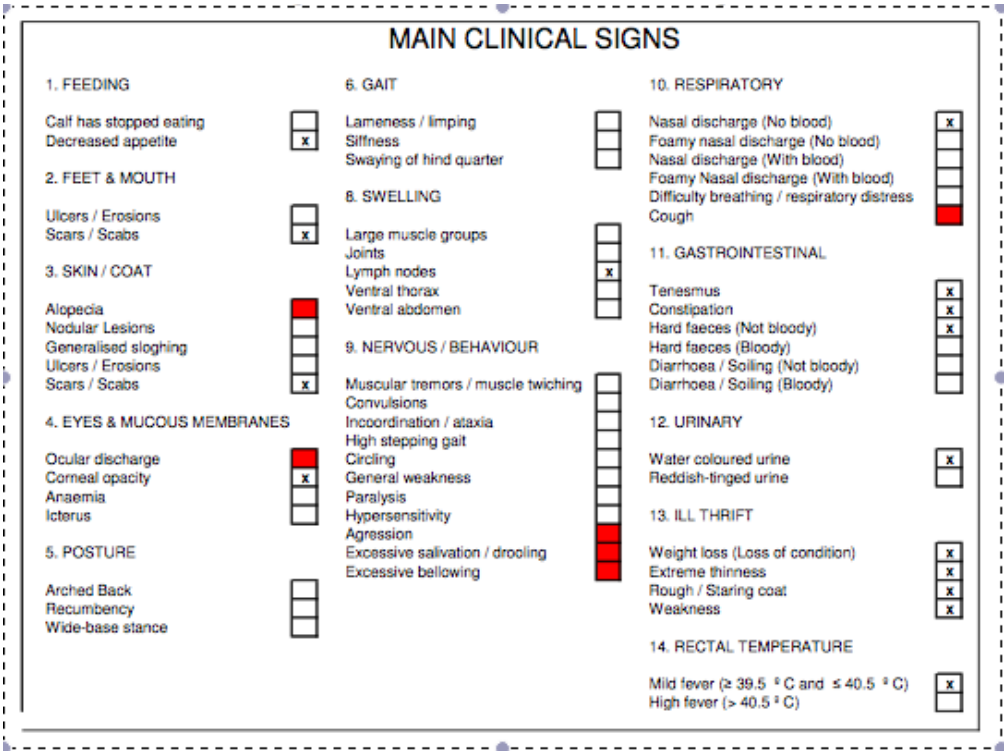

Figure 2.4: Aide Memoire card used by AHAs for assessing whether a visit to a calf is a clinical episode. To be used with figure 2.5.

Clinical signs which are highlighted with an 'x' cannot be regarded as a 'clinical episode' per se, but need to be accompanied by other signs of illness in order to be considered as clinical episodes (and hence reported to the IDEAL veterinarians). This is particularly true for lymph node enlargement, or ill thrift, which should only be reported if accompanied by:

- Anaemia (with or without other clinical signs) or
- High fever (>40.5ºC) ; (with or without other clinical signs [e.g. ill thrift])
- Other signs not marked with an 'x'

Clinical signs highlighted in red MAY constitute a clinical episode 'on their own' depending on the context. For example, aggression would not be reported if the animal has 'always' had an aggressive temper. However, you would regard aggressiveness as a sign of illness if this was the result of a sudden/dramatic behavioural change. You would not report focal alopecia, but you would report widespread alopecia specially if accompanied by thickening of the skin. Similarly, you wouldn't report mild ocular discharge in the absence of other signs of illness, but you would report severe mucopurulent ocular discharge even in the absence of other clinical signs. You would not report light coughing unless this is accompanied by - for example - high fever (>40.5ºC), but you should report severe persistent coughing even in the absence of other signs.

Clinical signs that are not highlighted with 'x' or in red should always be regarded as a clinical episode and reported even in the absence of other clinical signs.

In summary, the following are the most important / common conditions that should be reported to IDEAL veterinarians:

- Every case with a very high fever (>40.5ºC).
- Anaemia (pale mucous membranes) with or without lymph node enlargement and with or without high fever.
- Icterus and other associate signs of illness such as dark-brown urine and inappetence.
- Calves with respiratory distress/very rapid breathing which may or may not have a mucopurulent nasal discharge and a high fever (these are case of pneumonia).
- Diarrhoea (with or without blood).
- Multifocal deep-seated nodular skin lesions: sometimes accompanied by exudation.
- Alopecia (widespread) accompanied by thickening of the skin.
- Hypersensitivity and other signs on central nervous system (CNS) involvement such as high stepping or abnormal gait, muscle twitching, blinking with the eyes, etc.
- Bloody or blood-tinged discharges from nose.
- Profuse foamy discharges from mouth and/or nose.
- Ulcerative lesions in mouth and/or feet.
- Severe mucopurolent ocular discharges.

Clinical signs / injuries that are not listed in Table 6. but which reflect a serious threat to the wellbeing of the calf should also be reported to IDEAL veterinarians ASAP.

Figure 2.5: Protocol used by AHAs for assessing whether a visit to a calf is a clinical episode

post-mortem was disposed of safely and prevented from entry to the food chain.

All results were recorded on a palm computer and in paper format and all samples were barcoded and scanned using the palm top computer. Photos were also given a unique sample number that was associated with the calf identity number. A report was written by the attending clinician following completion of both the examination and the diagnostic work at the field laboratory.

Diagnostic testing of post-mortem cases

Testing was carried out depending on availability of samples and according to clinical presentation of the case. Techniques completed prior to and able to be included in the post-mortem report were as follows;

- McMasters faecal egg count,
- Faecal sedimentation and flotation
- Baermann's technique for lung worm
- Ziehl-Nielsen staining of faecal smears
- Larvation and microscopic identification of larval species
- Sporulation of coccidial species
- Culture and basic typing of bacterial isolates from faecal material or swabs taken from tissues (aseptic technique practiced when appropriate).
- An automated haematological count and manual packed cell volume measurement on whole blood
- Measurement of total serum protein
- Examination for haemoparasites in the buffy coat and Giemsa stained blood smears, lymph node aspirates, and impression smears (usually the spleen or lymph nodes)
- Fungal culture (usually from deep skin scrapes)

One calf was suspected of suffering from rabies. For the safety of staff the head was removed from the calf, packaged, and transported to a specialist lab. No further examination was conducted on the remaining carcass.

2.2.6 Farmer feedback

A report was written for each farmer following the final visit to each calf. This contained information on the birth weight of the calf and the calf's weight on leaving the study, the major pathogens identified by the field laboratory in Busia (see below), and advice on any veterinary interventions deemed appropriate. This was delivered to the farmer five weeks after the calf leaving the study when the IDEAL teams were next in the sublocation.

2.3 Diagnostic testing

2.3.1 Diagnostic testing at the Busia laboratory

Faecal samples were examined for endoparasites according to methods in Hansen and Perry (1994). The techniques employed that are relevant to this thesis were McMasters for faecal egg and coccidia counts, Baermann for the isolation of lungworms, and larvation of faeces to identify the parasites to genus level. Refrigerated samples were used for McMasters, Baermann, and sedimentation techniques. The faecal samples kept at room temperature samples were used for faecal larvation and sporulation.

Blood smears were examined for haemoparasites. Thin smears were fixed using methanol and were stained using Giemsa. Thick smears were directly stained as no fixing was required. 100 fields were examined under an oil immersion lens. Haemoparasites present were identified to genus level. The intensity of infection was semi-quantified. Level 1 = one parasite or infected cell per two to ten fields. Level 2 = one to ten parasites or infected cells in every field. Level 3 = more than ten parasites or infected cell in every field.

Packed cell volume (the percentage of total blood volume comprised of erythrocytes) was calculated following centrifugation of microhaematocrit tubes containing whole blood. A microhaematocrit reader was used to calculate the percentage of blood volume made up of red blood cells.

Total serum protein was calculated for serum. This was taken from centrifuged clotted blood samples. The total serum protein was read from a spectrophotometer which measures the refraction of light in water caused by solutes.

An automated haematology count was calculated by a Sysmex haematology analyser. This was validated by Van Wyk, 2012.

2.3.2 ELISA testing and seroconversion for the tick borne diseases

Species specific antibody ELISAs for *Theileria parva*, *Theileria mutans*, *Anaplasma marginale*, and *Babesia bigemina* were carried out at a specialist laboratory based on methods described in Katende et al. (1998), Katende et al. (1990), Morzaria et al. (1999), and Tebele et al. (2000), and were optimised by the laboratory (ILRI). All samples were tested in duplicate and a mean percentage positivity (PP) was calculated (PP = percentage positivity of the optical density of the positive control). The cut-off established by the laboratory carrying out the testing was 20PP for both the *T. parva* and *T. mutans* ELISAs. For the *B. bigemina* and *A. marginale* ELISAs the standard cut-off was 15PP.

Our analysis required identification of the visit in which seroconversion was detected. It was not possible to use a single visit above the cut-off in calves that had circulating maternal antibody. Calves that had absorbed maternally derived antibody from colostrum had high titres for some time after birth, whether or not they had been exposed to the parasite. Therefore, it was necessary to define a rule to utilise the serial serology data to identify if and when a calf had been exposed to one of the above infections. The serial serology results for each calf were plotted and studied for a rising antibody titre (an increase in ELISA PP result from visit to visit) and for a sustained level of antibody increase after that rise. It was seen that for all but *T. mutans* the

population mean PP dropped to its lowest point at week 16 (also applied by Davison et al. (1999)). This was taken to represent the point at which the majority of the population no longer had significant concentrations of maternally derived antibody circulating. Several rules were defined and were applied to the serial serology data and a final rule was iteratively developed that most closely described the event of seroconversion compared to the opinion of a clinician. The rules were developed from different combinations of seroconversion rules used by others previously (Katende et al. 1998; Swai et al. 2007; Magona et al. 2008; Davison et al. 1999; Gitau et al. 2000). Gitau et al. (2000) censored all calves that appeared to seroconvert during the decline of maternal antibody and did not include these calves in calculation of incidence of *T. parva* exposure.

The rules investigated were as follows:

- **Rule A:** The point of seroconversion was defined as the first visit from week 16 (decline of maternal antibody (Davison et al. 1999)) where the ELISA PP was above the cut-off defined for the specific ELISA (>20PP for *T. parva* and *T. mutans* and >15PP for *A. marginale* and *B. bigemina*) (Katende et al. 1998; Swai et al. 2007).
- **Rule B:** The point of seroconversion was defined as the first of two consecutive weeks over the cut-off (Davison et al. 1999) from week 16.
- **Rule C:** The point of seroconversion was defined as any visit over the cut-off from week 16, or any rise in ELISA PP by more than the cut-off before week 16 (Magona et al. 2008).
- **Rule D:** The point of seroconversion was defined as the first of two consecutive weeks over the cut-off from week 16, or any rise in ELISA PP by more than the cut-off before week 16.
- **Rule E:** The point of seroconversion was defined as a rise of more than the cut-off from the previous to the next visit, with seroconversion defined as happening at the 2nd of the two visits (Magona et al. 2008).

Rules A and B missed several early seroconversion events. A clause demanding a rise by at least the cut-off prevented the detection of several calves with smaller

incremental but consistent rises in PP. Rules C and D did not account for the decay in maternal antibody. If calves had acquired antibody from colostrum, a reduction from peak PP at the recruitment visit was expected. Therefore, any rise could have indicated a seroconversion event.

Another rule (Rule F) was devised iteratively from studying the calf profiles to improve on the sensitivity and specificity problems with rules A-E. This method identified the age at seroconversion using a moving window rule relying on three consecutive visits (A, B, and C) and agreement to three stipulations:

- The PP at visit B had to be higher than the standard cut-off. This was to ensure that the increase seen was not due to non-specific reaction. Some development of the dye can occur in the absence of specific antibody.
- The PP at visit B had to be more than the PP at visit A. This described a rising titre and allowed the potential for early seroconversions to be detected while levels of maternal antibody were still high.
- The PP at visit C had to be at least 5PP higher than at visit A. This was to ensure that the antibody rise was sustained above maternal antibody decay levels and the high PP at visit B was not a spurious result. Several different values were tried and calf serology profiles were inspected. The author found that an increase of 5 was found to best differentiate profiles that indicated seroconversion from those with a single high spurious result

As with results from all diagnostic tests it is possible for this rule to misclassify individuals. However, it was found to be the best solution following detailed examination of serological profiles and to agree very well with clinical opinion. For examples of serology profiles from calves see appendix G.

2.3.3 DNA extraction

DNA was extracted from whole blood samples for use in polymerase chain reactions (PCR) diagnostic tests and for the reverse line blot (RLB).

DNA was extracted using Qiagen DNeasy DNA extraction kit. Whole blood samples

were thawed in a water bath set at 37^0C. 200μL of whole blood, 20μL of proteinase K enzyme, and 20μL of RNase A enzyme were added to the supplied microfuge tube. The tube was vortexed and incubated for 2 minutes. 200μL of genomic lysis buffer was added to the sample, the tube was vortexed and incubated for 10 minutes at 55^0C. 200μL of ethanol was added to the sample, the sample was vortexed, and decanted to a spin column. The sample was then centrifuged at 10'000g for 1 minute. The spin column was placed in a clean tube and 500μL of wash buffer was added to the spin column. The sample was centrifuged again at 10'000g for 1 minute. The tube was discarded and the column retained and placed in a new tube. 500μL of a second wash buffer was added to the tube and again the tube was centrifuged. The tube was again placed in a clean tube. This tube was the final sample storage tube and was labelled with the sample ID number. 75μL of genomic elution buffer was added onto the filter in the column, and the sample was allowed to sit at room temperature for 1 minute. The column was centrifuged over the final sample tube. This elution, incubation, and centrifugation step was repeated with a further 75μL. The final sample was split across two sample tubes with identical labels, and both were frozen at -80^0C until needed.

2.3.4 Reverse line blot (RLB) for tick borne diseases

The reverse line blot testing was carried out at the Onderstepoort Veterinary Institute, University of Pretoria. *Theileria* and *Babesia* species were amplified using $RLBF_2$ (forward) and $RLBR_2$ (reverse) primers. *Ehrlichia* and *Anaplasma* species were amplified using Ehr-F (forward) and Ehr-R (reverse) primers. 5μL of DNA template (5-100 ng DNA) was added to PCR mastermix, and 25μL of PCR product was loaded to the RLB membrane. The general methods for RLB are described in chapter 1 in section 1.2.7.

2.3.5 Diagnosing the cause of death

Diagnoses of death was carried out by an expert panel. A meeting was convened of experts that included those who conducted the majority of the post-mortems on the study calves, pathologists, and cattle tropical disease specialists. The panel made a

decision on diagnosis following examination of all available information for each calf. The following testing was carried out following completion of the project and was available to the expert panel (see below) when making decisions about cause of death. Unless otherwise stated the testing was carried out at ILRI, Nairobi.

- The history of the calf recorded in routine and extra clinical visits (available to attending clinician).
- Diagnostic information from the field laboratory (available to attending clinician).
- Tick borne disease ELISA results and age at seroconversion.
- Photographs taken at the time of the post-mortem examination (available to attending clinician).
- A full-hand report written by the attending veterinarian.
- The histological report and diagnosis (Onderstepoort Veterinary Research Centre in Pretoria, South Africa).
- p104 PCR for *Theileria parva*. Sample from last visit prior to death (Odongo et al. 2010) (ILRI).
- Reverse line blot (RLB) testing results (Bekker et al. 2002) (Onderstepoort Veterinary Research Centre in Pretoria, South Africa).
- Real-time PCR assay for *Ehrlichia ruminatum* (Steyn et al. 2008) (Onderstepoort Veterinary Research Centre in Pretoria, South Africa) .
- ITS PCR for *Trypanosoma* spp. (Cox et al. 2005) (ILRI).
- PCR for Malignant Catharrhal Fever, and lumpy skin disease (the Institute for Animal Health, Pirbright, UK)
- Electron microscopic examination for viral particles and immune staining of tissues (Onderstepoort Veterinary Research Centre in Pretoria, South Africa).
- Toxicology (Onderstepoort Veterinary Research Centre in Pretoria, South Africa).

Each calf was attributed a primary cause of death and where necessary a contributing cause of death. Sometimes a diagnosis was not possible, either due to an incomplete set of samples, or because the clinical signs recorded were not able to be assigned to a particular cause. Further testing was indicated in some cases following the first round of examination or on recommendation of the pathologist. These tests were carried out after the initial meeting and the expert panel met for a second time at a later date with more information to attempt to diagnose further deaths. In cases where no specific cause was able to be identified the panel attempted to describe the death as infectious or non-infectious. For those calves that were euthanised the primary cause of death assigned to these cases was the cause that was believed by the panel to have triggered the euthanasia.

2.4 Statistical analysis

This section describes those statistical techniques that were used extensively throughout the thesis. Those methods used in a single chapter are described in that chapter.

2.4.1 Data reduction methods

Data reduction methods offer a way of reducing high dimensional data to fewer dimensions, to allow exploration and description of those complicated data sets. The variables are reduced to a smaller number of synthetic and independent variables that explain the maximum amount of variation in the data as possible. These variables, or dimensions, describe groups of interelated characteristics that tend to co-occur. They may be used to describe characteristics of individuals or groups of individuals in the data set, and can be very useful for modelling, especially where the number of parameters measured exceeds the number of individuals in the sample (Manly 2005; Ringnér 2008; Husson et al. 2011).

Three different data reduction techniques were used in this thesis. Continuous data were analysed using principal components analysis (PCA). Categorical data were

analysed using multiple correspondence analysis (MCA), and factor analysis for mixed data (Analyse Factorielle des Donnees Mixtes (AFDM)) was used when a mix of both categorical and continuous parameters were present. All these analyses were carried out in the package *FactoMineR* (Husson et al. 2010), in R (R Development Core Team 2010).

These techniques were used in this thesis to allow the visualisation of high dimensional data, to examine the relationship between variables within individuals with different outcomes.

Principal components analysis (PCA)

Principal components analysis reduces continuous measures to a small number of principal components which are linear combinations of the original variables (Ringnér 2008). It reduces high dimensional data to orthogonal components. The first principal component describes the axis of most variation, with each subsequent axis explaining the maximum of the remaining variation. It can allow the covariance of several continuous measures and the inter-relationships between different parameters to be visualised. It does not necessarily separate sub-groups within samples (Ringnér 2008). However, the output from PCA describes how much each of the original variables contributes to each of the summary components. PCA was carried out using the function *PCA* from the package *FactoMineR* (Husson et al. 2010). The function allows the incorporation of both quantitative and qualitative supplementary variables. These supplementary variables are not used to calculate the components, but allow the association between the components and these supplementary variables (for example, outcome) to be examined.

Multiple correspondance analysis (MCA)

Multiple correspondence analysis is a method for data reduction for categorical data. It maps high dimensional categorical variables onto a reduced number of continuous dimensions. It provides plots that are functionally similar to PCA. Everitt et al. (2001) described MCA as “a method for simultaneously assigning a scale to rows and a

separate scale to columns so as to maximise the correlation between the two scales. ... a technique for displaying multivariate categorical data graphically, by deriving coordinates .., which may then be plotted to display the pattern of association between variables graphically."

MCA was carried out using the function *MCA* from the package *FactoMineR* (Husson et al. 2010).

Rare variables in columns have a greater influence on the calculated chi-squared distances compared to more common ones (Everitt et al. 2001). However, the function *MCA* manages and scales according to how common variables are in the data set. Missing data are managed by insertion of the mean value.

Factor analysis for mixed data (AFDM)

AFDM allows the inclusion of both categorical and continuous variables into a single analysis (Bertrand et al. 2007; Fournié et al. 2012). Continuous variables are adjusted to have a mean of zero and a variance of one. Again, AFDM provides plots that are functionally similar to those from PCA. AFDM was carried out using the function *AFDM* in the package *FactoMineR* (Husson et al. 2010)

2.4.2 Logistic regression models

Logistic regression is a form of generalised linear modelling, that allows prediction of a binary outcome. The model estimates the probability of the outcome (disease / infection) given the covariate pattern and continuous predictors (the exposures to which the individual is exposed). Categorical and continuous variables are used to generate a linear predictor, which is then transformed to the probability scale using the logit transform. This transforms a predictor that can vary from -∞ and +∞ to a value that is restricted between zero or one (Hilbe 2009).

The form of the logistic model is:

$logit(Y) = \beta_0 + \beta_1 \mathrm{X}_1 + \beta_2 \mathrm{X}_2 + ... + \beta_i \mathrm{X}_i + \epsilon$

where exp(β) gives the odds ratio for a unit change in the predictor variable 'X' and where ϵ is the error term (Hosmer and Lemeshow 2000).

It is an assumption of the model that continuous variables are linear in their relationship to $logit(Y)$. If this is not the case continuous variables need to be categorised for inclusion in the model. The relationship between continuous variables and $logit(Y)$ can be investigated by categorising the variable, plotting the estimates in their logical order, and examining the change in $logit(Y)$. Violation of linearity can be tested using the Box-Tidwell test (Hilbe 2009), where $logit(Y)$ is modelled by an interaction between the continuous variable and the log of that variable. If the interaction term is statistically significant this suggests that linearity is violated.

The ability of the models to differentiate between outcomes using the incorporated risk factors was assessed using receiver operating characteristic (ROC) curves. This was carried out using the *lroc* function which is part of the *epicalc* package (Chongsuvivatwong 2010) in R. ROC curves plot the sensitivity against the false positive rate (1-specificity). Points laying above a diagnonal line with an intercept of zero and a gradient of one denote the test is performing better than chance alone (Dohoo et al. 2009). This can be applied to logistic regression models to assess model performance. The larger the proportional area under the curve, the better the model predicts the outcome overall. An area of more than 0.5 denotes a model that predicts the outcome better than chance alone.

2.4.3 Random effects models

Random effects models are a method for accounting for hierarchical structure in data. They are applied when data contains repeated measures (for example several data points from a single individual, or herd). They reduce the over-estimation of sample size, and correct the uncertainty intervals. Calves in the IDEAL cohort were randomly recruited from sublocations that were randomly selected from all those eligible within the study area. The selection of sublocations was stratified by AEZ to ensure that geographical variation was equally sampled. Calves were therefore geographically clustered. Calves closer together from within one sublocation were likely to be more

similar to their neighbours than calves from other sublocations. Some of this variation will have been measurable and recorded (such as difference in vegetation density), but some of the variation will have remained unexplained (Hilbe 2009). The specific sublocations selected were not of inherent interest, but were representative of the study area as a whole. Inclusion of the sublocations as random effects allowed results to be considered as representative of the study area rather than only for those randomly selected sublocations. It allowed for degrees of freedom to be conserved as it was not required to include sublocation as a fixed effect, and it helped to avoid over confidence in the model coefficient estimates. Sublocation was included as a random intercept and was specified to be normally distributed. The form of the logistic regression equation including random effects is: $logit(Y) = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + ... + \beta_i X_i + \gamma_i + \epsilon_i$ where γ is the random covariate.

The random effects were examined by comparing the within sublocation variability to the between sublocation variability using the following equation:

$\rho = \frac{\sigma_v^2}{\sigma_v^2 + \sigma_\epsilon^2}$

where σ_v^2 was the sublocation (random effect) level variation and σ_ϵ^2 was the standard deviation of the error term. ρ is the residual intraclass correlation coefficient and when close to zero the variation accounted for by the random effects was very small.

Logistic regression was carried out in R (R Development Core Team 2010) using the function *lmer* from the package *lme4* (Bates and Maechler 2010). This package allows the fitting of general linear mixed effects models with random effects.

2.4.4 Analysis of longitudinal studies

Survival analysis

Survival analysis analyses time to event data to calculate a probability per unit time of that event occurring. The hazard function gives the potential per unit time for an event to occur:

$h(t) = \underline{lim}\,\Delta \rightarrow \mathrm{P}(\mathrm{t} \leq T < t + \Delta t | T \leq t)\,\Delta t$

where $P(t \leq T < t + \Delta t | T \leq t)$ is the conditional probability, t is time, T is the survival time, and Δt is a small time interval (Kleinbaum 1996).

Survival analysis was used to estimate both the median survival times to an event (e.g seroconversion or death) and the probability of event occurrence by one year. This method accounted for the time at risk contributed by censored individuals. The analysis was carried out using the functions *Surv* and *survfit* from the package *survival* (Therneau and Lumley 2010) in R (R Development Core Team 2010).

Time discrete hazard analysis

The IDEAL data were mainly collected in discrete time at 5 weekly routine visits. Only mortality was systematically recorded in continuous time (post-mortem visits happened outside of this schedule in response to death of calves). Data collected in discrete time is well suited to analysis using time discrete hazard analysis (TDHA) (Singer and Willett 2003), with hazard as the risk of an event occurrence in a discrete time period *j*. This can be expressed as:

$\hat{h}(t_j)$ = Number Of Events$_j$ / Number At Risk$_j$

Discrete time hazard is "the conditional probability that individual *i* will experience the event in time period *j*, given that he or she did not experience it in any earlier time period" (Singer and Willett 2003), or:

$h(t_{ij})$ = Pr[T$_i$ = j|T$_i \geq j$]

where T$_i$ is the time period in which individual *i* experiences an event, $Pr[T_i = j]$ is the probability that individual *i* will experience the event of interest in time period *j*, and $Pr[T_i$ = j | T$_i \geq j]$ is the conditional probability that the event has not yet happened, and will either happen now or at some point in the future.

Calculation of the hazard of an event and plotting that hazard over time allows periods of relatively higher or lower risks to be identified.

The standard error on the hazard at a discrete time point is:

$se(\hat{h}(t_j)) = \hat{h}(t_j)(1 - \hat{h}(t_j))/NumberAtRisk_j$

It is possible to add independent variables into a time discrete hazard model and to estimate hazard ratios for those variables. The hazard ratios are estimated using a logistic regression model.

$logit(h(t_{ij}))$ = [$\alpha_1 D_{1ij}$ + α_2 D_{2ij} + $\cdots$ + $\alpha_J D_{Jij}$] + [β_1 X_{1ij} + β_2 X_{2ij} + $\cdots$ + β_P X_{Pij}] + ϵ_{ij}

where D is a time indicator for the variable and X is a predictor variable, $logit(h(t_{ij})$ = $\alpha)$ represents the base hazard and β the proportional change in that hazard according to the combination of the predictors X. The assumptions of the model in this form are that the change in hazard is proportional over all time periods, and that continuous predictors vary linearly with logit($h(t_{ij})$).

The hazard is derived from the estimate of the logistic regression model:

$h(t_{ij}) = \frac{1}{1+e^{-z}}$ where z is the linear predictor.

Random effects for both calf and sublocation were included in models to account for repeated measures (several visits across time to the same calf) and the geographically clustered sampling on sublocation (see above).

2.4.5 Model building and selection of variables

Throughout this thesis a standard model selection method was used for selection of the final multivariable model. A list of biologically plausible variables was selected from all those available. Time dependent variables were included as appropriate. Univariable logistic regression models were used to screen all variables in this list for association with the outcome of interest. Random effects were included as appropriate. All continuous variables were checked for their relationship with the $logit$(predicted outcome) and managed as described above. All those variables with a p value of <0.25 were carried forward to the multivariable model building stage. All these variables were added to a maximal model and backward selection was carried out. The variable with the highest p value was removed and the model re-estimated and this step repeated until all variables in the model were significant (p value of <0.05). All previously excluded variables were then added back into the model in turn. Likelihood

ratio tests were carried out to assess model fit. This was carried out using the function *anova()* in R. The Akaike's Information Criterion (AIC) was used to assess and compare models to decide on the final set of variables to include (Hilbe 2009). This is a parameter of model fit that corrects for the number of parameters in the model. Any reduction in AIC denotes a better model fit. AIC is estimated as part of the *lmer* function. AIC comparison requires the compared models to contain the same individuals. Any calves that had missing data for the variables of interest were excluded for the final stages of model comparison.

2.5 Investigation of effects on calf outcome of being a member of the IDEAL cohort

2.5.1 Introduction

The IDEAL study protocols aimed to maintain the recruited calves under as natural conditions as was possible to enable conclusions from the study to be generalised. However, to reduce confounding factors farmers were requested not to treat their calves. In addition, the regime of visits was intensive; homesteads were visited every five weeks by IDEAL staff members and every week by a local project contact (often a local government veterinary office representative or private operative of a local agroveterinary business); an unusual level of contact with veterinary professionals in this region.

A small validation study was carried out to investigate whether the activities of the study (restriction of treatment and frequent visits) affected the outcomes of the IDEAL cohort calves compared to calves living in the study area, but not recruited to the study. The weight and the PCV % of unexposed calves were measured and were compared to the weight and PCV % of IDEAL cohort exposed calves at their final visit.

2.5.2 Materials and methods

All calf births that occurred in the sublocations selected for the study were reported to the IDEAL office. However, only a random sample of these reported calves were recruited. This left an excess of calves that had the potential to have been recruited to the study, who had a known date of birth, and contact details, but had not been exposed to the IDEAL project. It was possible to visit the calves that were not recruited as they reached one year old and record firstly whether they were still in the homestead of birth and if not why not, and if they were present their weight, and their PCV. This allowed these outcomes to be compared between IDEAL (exposed) and non IDEAL calves (unexposed) at one year old. Due to the number of reported calves and the resources and time available it was decided to aim to sample five calves in every sublocation

giving a total of 100 calves. These calves will be referred to as the unexposed cohort.

Calves to be recruited to the unexposed cohort had to have been reported to the main study during the two years of IDEAL project recruitment, the calves needed to adhere to the IDEAL project inclusion criteria, and had to have reached 51 weeks old within a four week period either side of the proposed visits dates to the unexposed calves. This allowed the spread of ages in the unexposed calves to match that of the IDEAL cohort while maximising the number of calves available. A list of calves that had the potential to be recruited into the unexposed cohort was generated from the Reports Database (a Microsoft Access database). This was populated during the IDEAL project recruitment and collected together all the calf birth reports and the homestead contact details as they were received at the IDEAL office. Recorded in this database was whether a homestead had been visited and recruited to the IDEAL cohort, had failed the recruitment criteria, or had refused to participate. Any calves that had not been randomly selected and had not been excluded were available for inclusion in the unexposed study.

The target was to randomly select five calves from each sublocation from this list of unvisited calves. The contact details for these calves were passed onto a contact person in each sublocation (the chief or village elder). If 5 or fewer calves were available all were selected. This was done 5 weeks in advance of the expected visit date to enable the farmers willing to participate to make their calves available. If farms refused permission or the calf had left the farm for a reason other than death, and if excess calves were available then another calf was randomly selected.

This validation study was carried out in July and August 2009, and January to April 2010. For logistical reasons, visits to the unexposed calves were made on the same day as visits to the IDEAL calves in each sublocation. As an incentive for participation by unexposed cohort farms veterinary health advice was given at the time of the visit, and samples were taken to assess the haemoparasite load of the calf. This data, along with the calf's weight and packed cell volume (PCV) was complied into a report and was delivered to the farmer the next time IDEAL staff visited the sublocation.

Homesteads were visited, consent obtained from the most senior member of the household, and a questionnaire conducted. This was a shortened version of the normal

routine visit questionnaire (appendix E). The questionnaire was completed by an IDEAL member of staff through interview of the best informed household member present. The full questionnaire was conducted if the calf was alive and present. If the calf was reported as dead before 1 year old a shortened version was carried out.

Sampling was carried out as for the protocols described for the main study. Questionnaire and lab data were transposed to a Microsoft Access database.

A mixed effects linear model was fitted with a random effect for sublocation using the function *lmer* from the package *lme4* (Bates and Maechler 2010) in R (R Development Core Team 2010). Analysis of variance (anova) was used to assess whether a variable significantly contributed to model fit. This was carried out using the function *anova()* as part of the standard *stats* package in R.

2.5.3 Results

Table 2.2: Sublocation identification numbers, agroecological zones, and number of IDEAL calves reaching the final visit and the number of alive unexposed calves visited at one year old.

Sublocation name	Agroecological zone	No. IDEAL calves	No. control calves
East Siboti	UM3	20	13
Kidera	UM3	24	9
Kokare	UM3	24	2
Mabusi	LM2 middle	25	9
Kamunoit	LM2 middle	25	2
Karisa	LM2 middle	24	0
Otimong	LM1	22	6
Igero	LM1	25	1
Bulwani	LM1	23	1
Bukati	LM1	27	5
Ikonzo	LM1	24	0
Bumala A	LM1	15	1
Yiro West	LM1	21	3
Simur East	LM1	25	3
Namboboto	LM2 south	24	1
Ojwando B	LM2 south	21	2
Kodiere	LM2 south	23	6
Luanda	LM3	26	1
Bujwanga	LM3	21	2
Magombe East	LM3	16	2
TOTAL		455	69

A total of 69 calves were recruited to the unexposed cohort. This was 31 calves below the target number. One farmer refused to participate. No unexposed calves were available from Karisa or Ikonzo (table 2.2). Therefore, the data from IDEAL calves from Karisa and Ikonzo was excluded from the analysis. More than 5 calves were visited in several sublocations in order to increase the sample size. The inability to recruit the required number of calves was due to lower reporting rates in some sublocations when compared to other (see figure 2.6). It is unknown whether these differences were due to birth rates or to the proportion of births reported by local contacts. Eight of the 69 homesteads visited reported that their unexposed calf had died in its first year of life.

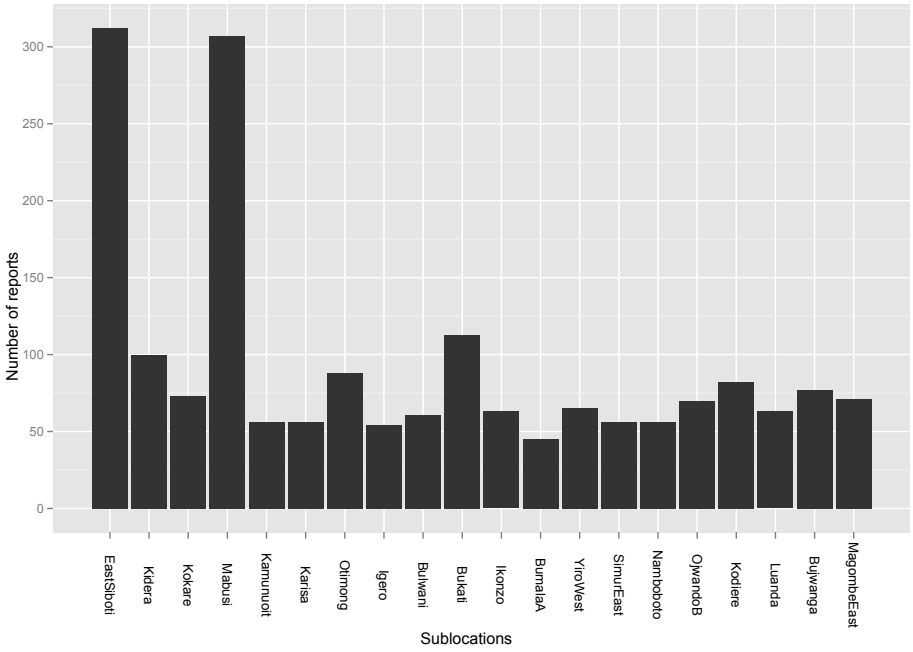

Figure 2.6: Barchart demonstrating the difference in reporting rates between sublocations for the IDEAL study. It can be seen that the sublocations in which excess unexposed calves were recruited, East Siboti and Mabusi, had much higher levels of reporting compared to other sublocations. A large number of sublocations only had one calf not recruited for every calf recruited (average of 28 calves recruited and just over 50 calves reported)

Mixed effects linear regression with a random effect for sublocation was used to investigate whether there was an association between weight or PCV% at one year and group membership (IDEAL exposed and unexposed calves). Age and sex were included in the models to control for these factors.

The weights of the calves at one year old from the IDEAL cohort and the unexposed cohort are summarised in figure 2.7. The mean weight in the IDEAL cohort at the final visit was 65.2Kg (range: 29 - 144Kg). The mean weight in the unexposed cohort was 67.9Kg (range: 28.5 - 166.5Kg). There is no evidence from this plot that the mean weight of the IDEAL calves at one year old were different to their unexposed contemporaries. The linear mixed effects model estimates are summarised in table 2.3. The addition of group membership to the model for the prediction of weight did not significantly improve the fit of that model (p value = 0.298).

The packed cell volumes (PCV) of the calves at one year old from the IDEAL cohort and the unexposed cohort are summarised in figure 2.8. The mean PCV in the IDEAL cohort at the final visit was 27% (range:10 - 42%). The mean PCV in the unexposed cohort was 29% (range: 20 - 35%). There is evidence from this plot that the mean PCV of the IDEAL calves at one year old was lower than the unexposed calves. The linear mixed effects model estimates are summarised in table 2.4. The addition of group membership to the model for the prediction of PCV significantly improved the fit of that model (p value = 0.006)

Table 2.3: Summary of linear mixed model of calf weight at one year. SE = standard error. LCL = lower boundary of 95% confidence interval. UCL = upper boundary of 95% confidence interval.

	Estimate	SE	t value	LCL	UCL
(Intercept)	33.587	38.523	0.872	-46.089	104.917
Calf sex male	-	-	-	-	
Calf sex female	-3.213	1.548	-2.076	-2.966	3.102
Age (days)	0.101	0.109	0.928	-0.212	0.214
Unexposed calves	-	-	-	-	-
IDEAL calves	-2.543	2.459	-1.034	-7.715	1.925

2.5.4 Conclusions

A key outcome measure, body weight at 1 year old, was not found to be significantly different in those calves recruited to the IDEAL study and those that had the potential to be recruited to the study but were not (unexposed cohort). However, PCV was found to be significantly lower in the IDEAL calves when compared to those calves not

Table 2.4: Summary of linear mixed model of calf PCV% at one year. SE = standard error. LCL = lower boundary of 95% confidence interval. UCL = upper boundary of 95% confidence interval.

	Estimate	SE	t value	LCL	UCL
(Intercept)	29.414	8.950	3.286	11.872	46.956
Calf sex male	-	-	-	-	-
Calf sex female	0.068	0.360	0.189	-0.638	0.775
Age (days)	0.001	0.025	0.047	-0.048	0.051
Unexposed calves	-	-	-	-	-
IDEAL calves	-2.895	0.571	-5.070	-4.014	-1.776

recruited to the study. The mean difference in the IDEAL calves was -2%, but the group mean was 27% which is above what is usually considered to be anaemic (Schlam 2000). The biological significance of this difference is difficult to ascertain. A fall in PCV could be due to a number of causes. Some possible explanations in this context are haemoparasites, *Haemonchus* infestation, or in the case of the IDEAL calves, the regular removal of blood. However, the amount taken from calves at each routine visit was very small (20-30ml) and the time between visits was long enough for calf to replace this loss. The IDEAL calves were not able to be treated, so farmers may not have responded and treated calves that appeared to be suffering from haemonchosis or haemoparasitic disease. However, levels of veterinary intervention in this region are known to be very low. It could be said that the restriction on the use of endoparasitic treatments on the IDEAL calves caused this group to have a higher burden of blood sucking *Haemonchus* leading to the lowered PCV. This assumes that farmers treated those calves not recruited to the study. The lowered PCv could also have been caused by the repeated bleeding of the cohort calves. The amount of blood taken at each visit was calculated to be able to be replaced by calves over the 5-week inter-visit period. However, it may be that the combination of parasitic blood loss with the regular bleeding prevented calves from being able to compensate for the loss of blood.

It should be noted that the unexposed calves were recruited from those born over a shorter period of time. They were not sampled from across the 2 year period of IDEAL recruitment. Season or period of birth was not accounted for in the models and this may have affected results.

In conclusion, this validation study provides evidence that the IDEAL study design

allowed participating calves to remain good representatives of the study population despite the intensive regime and restrictions in place. Any lowered PCV % did not appear to have led to a reduction in growth rate.

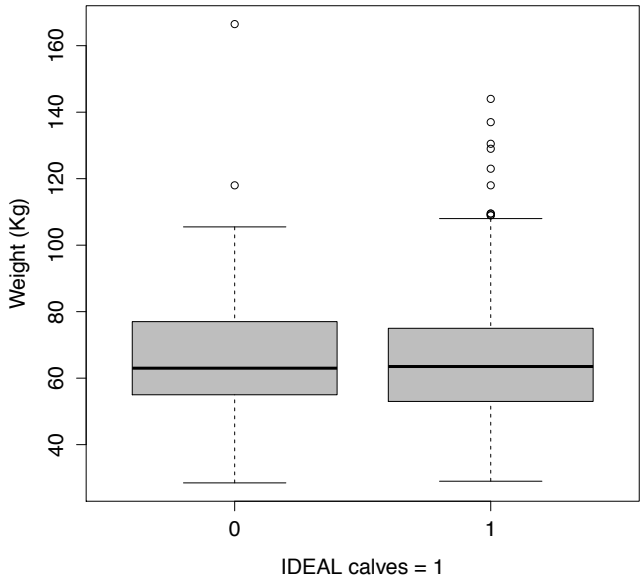

Figure 2.7: Boxplot summarising the weights of calves in the IDEAL and the unexposed cohort.

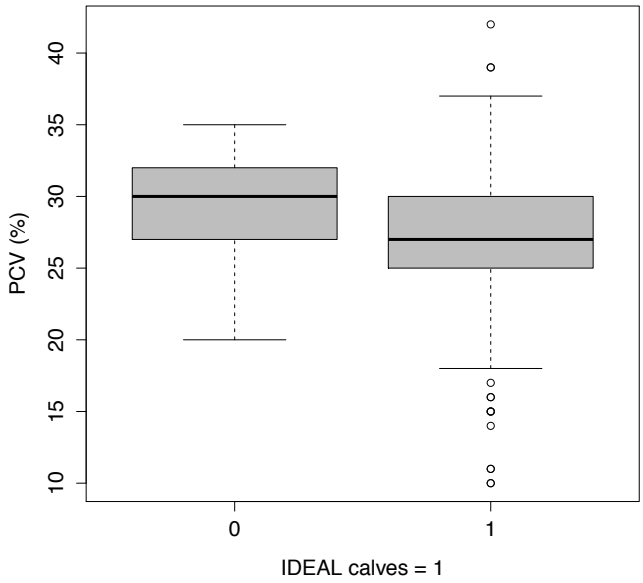

Figure 2.8: Boxplot summarising the PCV of calves in the IDEAL and the unexposed cohort.

Chapter 3

Theileria species and their vectors in the study cohort

Chapter abstract

Theileria species are widespread in Western Kenya. 362 of the 548 calves in the study cohort were classified as having seroconverted to *Theileria parva* and 381 to *Theileria mutans* before 1 year old. This chapter describes *Theileria* species in the study population, discusses the dynamics of infection, and seeks environmental and calf level risk factors associated with the age at which seroconversion occurred.

Results show that the tick vector of *T. parva*, *Rhipicephalus appendiculatus*, had infected many of the calves shortly after birth. *T. mutans* was found to infect calves at a younger age than *T. parva*, and *Theileria* species were found together in individual calves dependent on a shared tick vector.

Data suggested that antibody levels declined quickly following first exposure to *T. mutans*, and that there was little evidence of boosting of antibody levels from persistent or re-infection. However, it is suggested that this may have been due to a mismatch between the antibody able to be detected by the species specific ELISA and that circulating in the calf.

Decreased elevation of the homestead and increased size of the herd were found to be significantly associated with an increased hazard of seroconversion to *T. parva*. However, there was little variation in the hazard of *T. mutans* captured across the study site.

Results from these analyses raise interesting questions about the co-occurrence of *T. parva*, *T. mutans*, and other species of *Theileria*, and their vectors. Further testing and analysis and validation of current diagnostic techniques will be required to confirm and expand on the current findings.

3.1 Introduction

Previous studies have investigated the prevalence of, and the risk factors associated with, infection with *T. parva* (Barnett 1957; Moll et al. 1984; Gitau et al. 1997, 1999, 2000; Maloo et al. 2001b; Gachohi et al. 2010)

Seroprevalence of *T. parva* has been reported to vary by agro-ecological zone (AEZ) (Maloo et al. 2001b) and herd management method (Gitau et al. 1999), with farms that practiced zero grazing and those that were at higher altitudes being reported to have been at decreased risk. The understanding of where, when, and in whom infection occurs is essential for both the development of effective intervention strategies, and their rational implementation. However, identifying infected individuals and herds correctly can be challenging.

The identification of *Theileria* infected or exposed animals has been carried out using a variety of methods including:

- identification of *Theileria* species by microscopic examination of blood smears (Barnett 1957; Moll et al. 1984; Maloo et al. 2001b).
- examination of a single serum sample by antibody ELISA and application of a cut-off (Gitau et al. 1997; Maloo et al. 2001b,a; Swai et al. 2007; Gachohi et al. 2010).
- identification of exposure through a rising antibody titre (Moll et al. 1984; Gitau et al. 1999).
- through the use of nucleic acid based tests (Morzaria et al. 1999; Gubbels et al. 1999; Bishop et al. 2001; Odongo et al. 2010; Thekisoe et al. 2010; Patel et al. 2011)
- identification of clinical cases (Barnett 1957; Moll et al. 1984; Latib et al. 1995; Gitau et al. 1999).

The above studies frequently employed more than one of the methods described above, possibly because each test was imperfect, or the results from each test answered a slightly different question.

This chapter investigates the different diagnostic tests used for identification of *Theileria* species in the IDEAL calves. Each different test was able to complement the results from other tests, and the comparative trends between *T. parva* and *T. mutans* were able to be described. Comparisons were also made with other tick borne diseases to further clarify findings. Spatial patterns of infection pressure were investigated as well as risk factors for exposure to *T. parva* and *T. mutans*.

3.2 Methods

3.2.1 IDEAL study design

The IDEAL study design and data collection methods are described in chapter 2.

3.2.2 Identification of infection

Theileria exposure or infection in the cohort calves was identified using five different methods:

- **Microscopy**. This method was able to identify parasites present in blood smears to genus level. It was carried out on smears from every 5-weekly routine visit (see chapter 2, section 2.2.3). Two different smear types were examined for each visit: one thick and one thin blood smear (chapter 2, section 2.3.1).
- **Serology**. Species specific ELISAs were carried out on serum samples taken from calves at each routine visit, and on samples taken from the dams at the recruitment visit. The test gave a percentage positivity, and this could be used either quantitatively as a proxy for antibody levels, or by applying a cut-off and defining the calves (or dams) as negative or positive for antibodies (chapter 2, section 2.3.2). Species specific ELISAs were carried out for *T. parva*, *T. mutans*, *A. marginale*, and *B. bigemina*, and although this chapter focuses on *Theileria*, results for all these species are presented for comparison in some cases.

- **Seroconversion**. This identified whether and by what age a calf was exposed to *T. parva* or *T. mutans* through the examination of serology results from consecutive visits. The method for defining the point of seroconversion is described in chapter 2, section 2.3.2).
- **Reverse line blot (RLB)**. RLB identifies all the tick borne diseases commonly found in Eastern and Southern Africa using a single diagnostic test. Results included here are those from the final visit to calves that survived to one year. The method for RLB is described in chapter 2, section 2.3.4.
- **East Coast Fever (ECF) death**. Infection with *T. parva* was identified for the first time at post-mortem examination in some calves(chapter 2, section 2.2.5).

3.2.3 Data analysis

All data extraction and analysis was conducted in R, version 2.15.1 (R Development Core Team 2010) using the in-built packages and *RMySql* (James and DebRoy 2012), *lme4* (Bates and Maechler 2010), *epicalc* (Chongsuvivatwong 2010), *xtable* (Dahl 2009), *reshape* (Wickham and Hadley 2007), *ggplot2* (Wickham 2009), *survey* (Lumley 2012), *FactoMineR* (Husson et al. 2010), *e1071* (Dimitriadou et al. 2011), and *survival* (Therneau and Lumley 2010).

Cohen's Kappa was used as a measurement of agreement between the results of diagnostic tests. The method calculates the amount of agreement between tests beyond that which would have been expected by chance alone (Dohoo et al. 2009). This was carried out using the function *classAgreement* from the package *e1071* (Dimitriadou et al. 2011) in R (R Development Core Team 2010).

Although similar numbers of dams were recruited from each sublocation, the sublocations had varying populations of adult breeding female cows, and prevalence estimates needed correction for that to ensure that sublocations with fewer animals were not over represented in estimates. The package *survey* (Lumley 2012) in R (R Development Core Team 2010) was used according to methods in Lumley (2004) to weight prevalence estimates by the number of breeding dams per sublocation.

Multiple correspondence analysis (MCA) was used to look at patterns in the co-infections of species of *Theileria* identified by RLB in the calves. MCA was carried out using the *FactoMineR* package (Husson et al. 2010). MCA is described in chapter 2, section 2.4.1. MCA was carried out with and without the rare species to investigate whether the influence of the uncommon species altered the interpretation.

Survival analysis was used to calculate the probability of infection by one year. Kaplan-Meier plots were used for visualisation. This was carried out according to the methodology described in Kleinbaum (1996) and using the *survival* package (Therneau and Lumley 2010) in R. This method is described in chapter 2 in section 2.4.4.

Time discrete hazard analysis (TDHA) was used to model the by-period hazard of seroconversion to *T. parva* and *T. mutans* according to the method described in chapter 2, section 2.4.4. All visits up to and including the week of seroconversion to either *T. mutans* or *T. parva* were included in the analyses. Those calves that died or left the study at one year before seroconverting were censored from their final visit.

Time-dependent and time-independent exposures were investigated for significant associations with the hazard of seroconversion. Model fitting methods were carried out as described in chapter 2, section 2.4.5. Random effects for calf and sublocation were included to account for repeated measures and study design. It was checked whether the random effect for sublocation improved model fit using a likelihood ratio test. A full list of variables tested for association are in appendix H.1.

3.3 Results and discussion

Samples from a total of 5337 routine visits to 548 calves were tested using the diagnostic testing methods described above.

3.3.1 Summary of results from microscopy

Of the 5337 routine visits carried out, only two visits had neither a thick nor a thin peripheral blood smear examined for blood borne parasites. Ten thin smears and 13

thick smears from routine visits failed and so did not have a result. Missing data points were generally due either to slides that did not stain or were damaged during the staining process. Blood smears were assessed for infection intensity of both schizonts and piroplasms. The life stages were recorded separately and it was possible to identify both in a single smear.

2747 of 5327 thin smears and 2516 of 5324 thick smears (52% and 47%) had *Theileria spp.* identified during examination, suggesting that examination of the thin smears was the more sensitive method. The two smear methods were compared and were tested for agreement using Cohen's Kappa (tables 3.1, 3.2 and 3.3). The agreement between the two methods for identification of *Theileria* piroplasms was very high, 0.62 and 0.69, respectively. The agreement was poorer for the identification of schizonts, with thin smears showing a higher sensitivity. The identification of schizonts is particularly useful in the diagnosis of clinical cases so this is of relevance for field diagnosis. A visit was recorded as positive by microscopy if either smear was recorded as positive for *Theileria* species. 3037 of 5335 visits (result from at least one of either thick or thin smear) were positive for *Theileria* spp. (57%).

The proportion of positive visits per calf was calculated. 22 calves (4%) had no *Theileria* spp. positive routine smears during their time in the study. Of those 22 calves, 20 of them died before one year old, ten of those from *T. parva* (ECF). The remaining two calves were censored after their routine visit at recruitment or visit week 16 because they were stolen or treated. Therefore all calves that reached one year old were recorded as positive for *Theileria* spp. on microscopy at least once during their time in the study. This is in agreement of the findings of Moll et al. (1984). The modal count of positive visits per calf was seven and the mean proportion of positive visits was 0.55 (see figure 3.1).

The youngest calf in which a *Theilieria* spp. positive smear was recorded was only three days old. *Theileria* piroplasms were observed in blood smears at the recruitment visit in nine calves (1.6%). In experimental infections, schizonts are first observed at five to 15 days following attachment of the tick and piroplasms at 10-25 days after attachment of the tick (Coetzer and Tustin 2004). It was not possible for a post-natal infection to have led to erythrocytic infection within seven days of birth by which age

Table 3.1: Summary of smear results from routine visits (thick and thin smear results for *Theiliera* spp.

		Thick Smear			
	Result	Theileria -ve	Theileria +ve	Not tested	Total
Thin Smear	Theileria -ve	2279	297	4	2580
	Theileria +ve	523	2217	7	2747
	Not tested	6	2	2	10
	Total	2808	2516	13	5337
	Cohen's Kappa	0.69			

Table 3.2: Summary of smear results from routine visits (thick and thin smear results for piroplasms for *Theiliera* spp.

		Thick Smear			
	Result	Piroplasm -ve	Piroplasm +ve	Not tested	Total
Thin Smear	Piroplasm -ve	2484	543	5	1032
	Piroplasm +ve	453	1836	6	2295
	Not tested	6	2	2	10
	Total	2943	2381	13	5337
	Cohen's Kappa	0.62			

Table 3.3: Summary of smear results from routine visits (thick and thin smear results for piroplasms for *Theiliera* spp.

		Thick Smear			
	Result	Schizont -ve	Schizont +ve	Not tested	Total
Thin Smear	Schizont -ve	4952	77	10	5039
	Schizont +ve	251	36	1	288
	Not tested	8	0	2	10
	Total	5211	113	13	5337
	Cohen's Kappa	0.15			

all recruitment visits had taken place. Trans-placental transmission of the parasite should be considered, but as this has not been reported before under field or controlled experimental conditions it seems an unlikely explanation. It could be that farmers reported that calves were younger than they truly were to enable their recruitment to the study (cash compensation was offered for participation in the study). However, care was taken by the field staff when examining both the dam and the calf and when interviewing members of the household to confirm the reported age. It is possible that these 9 results were false positives, and they were able to be spotted because of their implausibility. It should be considered that misclassifications may have happened at all ages but were not so easily identified. False positives may have occurred during the reading of the slide, or when the results were recorded. Slides were barcoded at the calf side and scanned into the calves' record. Therefore misidentification of slide to calf in the field would have been unlikely.

The oldest age at which a calf was first identified as *Theileria* positive by blood smear was 358 days old. The mean age at first *Theileria* spp. positive smear was 100 days, with a median age of 77 days. This was later than the ages reported by Moll et al. (1984).

Figure 3.2 summarises the dynamics of *Theileria* spp. by age as recorded by microscopic examination of blood smears. At all ages there was a higher proportion of visits positive for piroplasms compared to schizonts, as similarly observed by Moll et al. (1984). The proportion of positive visits increased from birth and then plateaued at week 41 after which 70 to 80% of visits were positive for the parasite; a very high level of infection. Piroplasms are always found in the circulation, whereas schizonts can be restricted to lymph tissue, especially following the initial peak of infection, so it is not surprising that piroplasms were recorded more commonly. It was also likely that many of the piroplasms were those of *T. mutans*, as the piroplasm is the dominant life stage for this species and have been observed to remain in the circulation for extended periods (Norval et al. 1992).

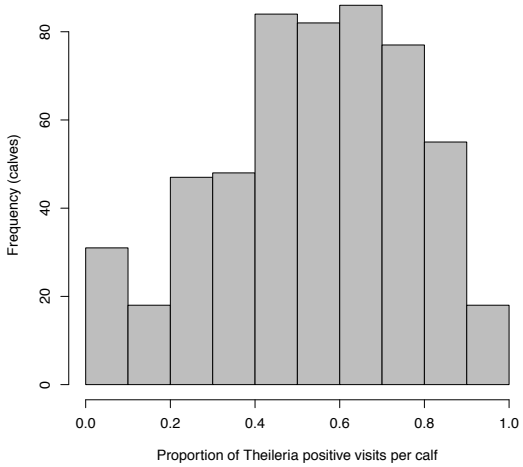

Figure 3.1: Summary of the proportion of visits per calf in which at least one of the thick or thin peripheral blood smears were positive for *Theileria* spp. by the number of calves.

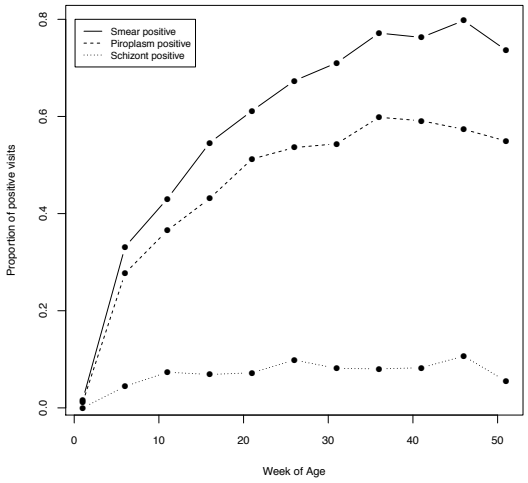

Figure 3.2: Proportion of visits positive by week of age. Summarised by those positive for *Theileria* spp., those positive for schizonts, and those positive for piroplasms.

3.3.2 Serological testing

81% of calves were classified as having maternally derived colostral *T. parva* antibodies at their recruitment visit, compared to 47% for *T. mutans*. 355 of 548 dams were classified as positive for *T. parva* at the recruitment visit (using the standard laboratory cut-off of >20PP = positive), 186 were positive for *T. mutans* antibody (>20PP = positive), 151 for *A. marginale* antibody (>15PP = positive), and 255 for *B. bigemina* antibody (>15PP = positive), giving a corrected prevalence for *T. parva* of 0.67 (95% CI: 0.61-0.72), for *T. mutans* of 0.35 (95% CI: 0.30-0.39), for *A. marginale* of 0.28 (95% CI: 0.24 - 0.32), and for *B. bigemina* of 0.48 (95% CI: 0.44 - 0.52).

The mean PP in the dams at recruitment for each species ELISA is plotted in figure 3.3 and can be compared to the mean PP in the calves at recruitment. In all cases the mean PP was lower for dams than calves. The relationship between individual calf and dam pairs can be seen in figure 3.4. The majority of calves had a higher PP at birth than their dams. In the pre-parturient period antibody production is believed to increase (Sasaki et al. 1976), but there is also an increase in the expression of the receptors in the lacteals that bind IgG (the predominant antibody type in bovine colostrum) in the period just before birth (Stelwagen et al. 2009). This increased production of antibody is unable to compensate for the large amounts of IgG actively bound and removed by receptors from the blood of the dam to the milk. This can lead to a periparturient drop in circulating antibody levels. As well as causing many of the calves to have higher antibody titres than their dams, this may also have reduced the apparent prevalence of exposure in the dams to below that of the general adult cattle population.

For a significant minority of calves, the levels of antibody in the calf was lower than that in the dams. The reason for this could be that these calves took less colostrum, or sucked too late (the ability to absorb antibodies through the intestine declines rapidly after birth), that the colostrum was of lower quality, or that the dams had such high levels of antibody that the uptake in the calf was saturated.

Following the recruitment visit, the mean PP for all 4 species declined as the levels of maternally derived antibody reduced in the calves' blood streams. Plots of *T. parva*, *A. marginale*, and *B. bigemina* show a decline in maternal antibody up until week 16 after which point the population mean antibody levels began to rise. Previous studies in

zebu calves observed maternal antibody to wane by 3 to 3.5 months of age (Moll et al. 1984; Gitau et al. 2000), slightly younger than observed here.

Following the decline in maternal antibody levels, the mean PP began to increase indicating exposure at the population level. For *A. marginale*, and *B. bigemina* this rise in mean antibody levels continued up to and including the final visit at 1 year old (week 51). For *T. parva* the levels had levelled out by one year old. For *T. mutans* the trend in antibody levels in the population was different. Fewer calves received maternally derived antibodies to *T. mutans* when compared to *T. parva*. The mean PP was at its lowest at week 6 and increased rapidly up until week 16 (the visit where the PP for all other species was at its lowest). After week 16 the antibody levels in the population declined gradually towards the final visit at one year. These plots would suggest that the infection pressure for *T. mutans* was very high leading to exposure in very young calves. This early exposure may have obscured the decline in maternal antibody levels.

3.3.3 Seroconversion

362 calves seroconverted to *T. parva*, 377 to *T. mutans*, 178 to *A. marginale*, and 117 to *B. bigemina*. The probability of an individual seroconverting by one year was 0.732 (0.689-0.769) for *T. parva*, 0.745 (0.703-0.781) for *T. mutans*, 0.362 (0.318-0.403) for *A. marginale*, and 0.238 (0.199 - 0.275) for *B. bigemina* (figure 3.5).

The median age at seroconversion to *T. parva* was 184 days, and to *T. mutans* was 129 days. Figure 3.6 allows comparison of the time discrete hazard of seroconversion through time and between species. *B. bigemina* was not included as the by visit hazard was very low over the first year of life. For *A. marginale* the hazard of seroconversion increased gradually over the year. However, for both *T. parva* and *T. mutans* the peak hazard was observed before one year old. For *T. parva* the hazard increased from week 6 onwards reaching a peak at week 26. Following this, the hazard generally declined with a small secondary increase at weeks 36 and 41.

For *T. mutans* the period of highest hazard was earlier, at week 11. This coincides with the increase in mean antibody levels in the population seen in figure 3.3. The trend was for calves to seroconvert to *T. mutans* before *T. parva* as can be seen from the median

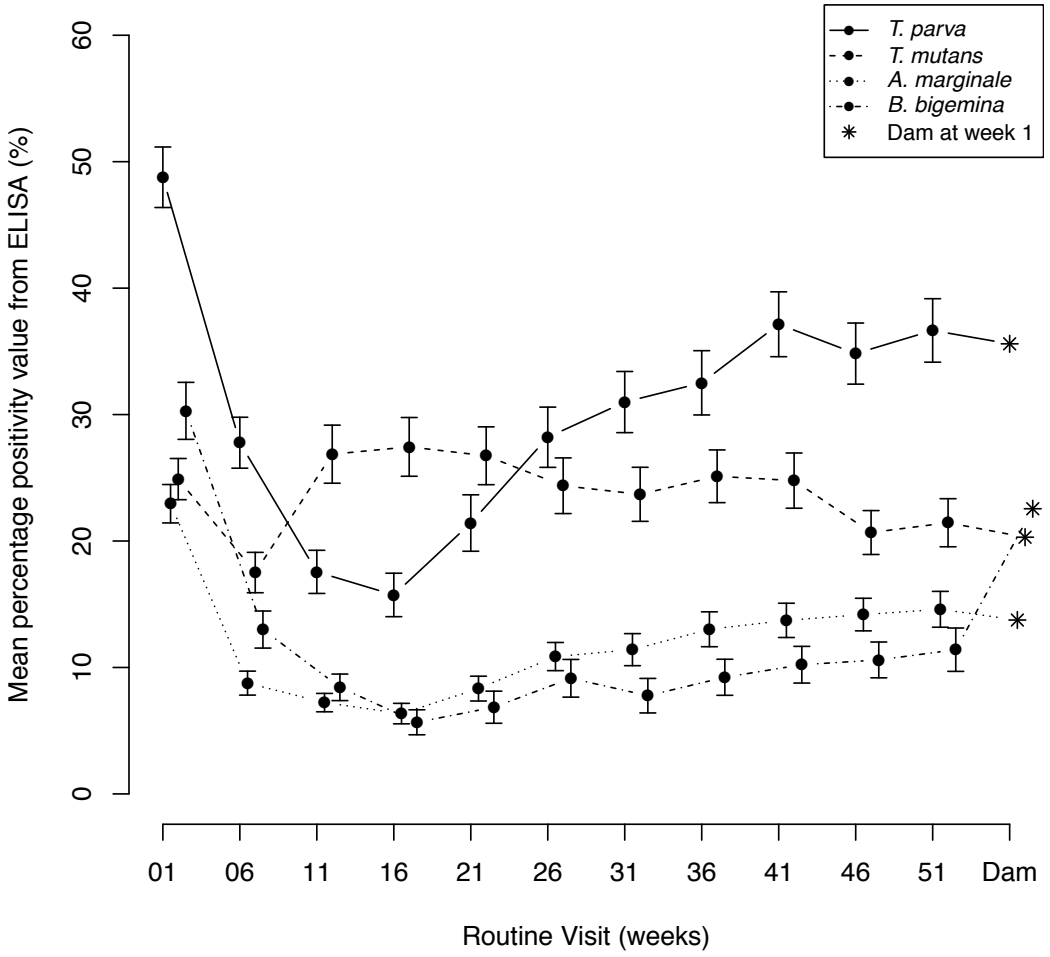

Figure 3.3: The mean ELISA PP by visit for each of the four tick borne disease species tested. The ELISA PP can be used as a proxy for antibody levels. The vertical bars represent 95% confidence intervals.

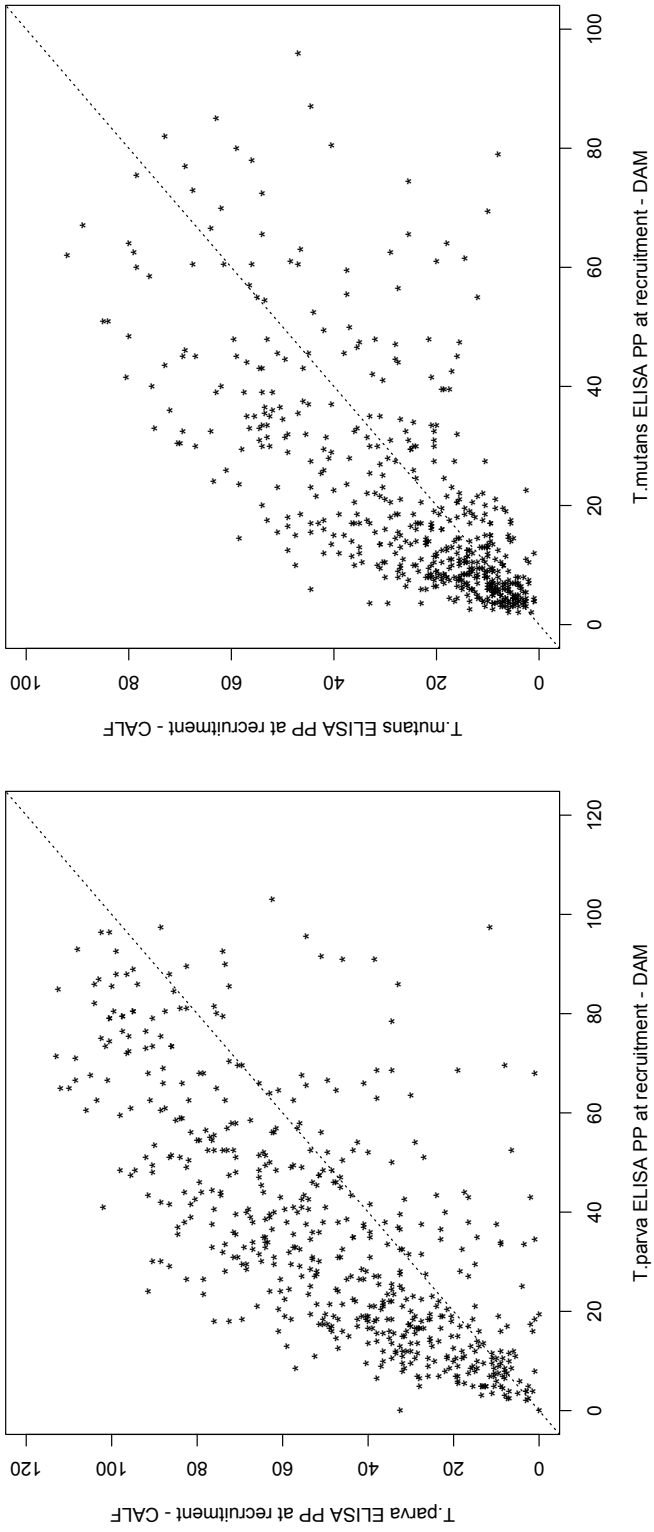

Figure 3.4: Comparison of the *Theileria parva* and *Theileria mutans* ELISA PP value for the calf and its dam at the recruitment visit.

ages at seroconversion (figure 3.5), and the high hazard periods (figure 3.6). However, there was no correlation between the age at which calves seroconverted to *T. parva* and *T. mutans* (figure 3.7). In other words, those calves that seroconverted at a very young age to *T. mutans* were not necessarily among the first calves to seroconvert to *T. parva*. Of the 276 calves that seroconverted to both *T. mutans* and *T. parva*, 182 were recorded as having seroconverted to *T. mutans* first, 50 calves to *T. parva* first, and 44 simultaneously to both (seroconversion occurred during the same 5 week inter-visit period).

Similar observations were made by Moll et al. (1984), who recorded that 83% of calves showed an active antibody response to *T. parva* and 98.8% to *T. mutans* in the first 6 months of life. Correspondingly, the active antibody response to *T. mutans* was generally seen to precede the response to *T. parva*. The mean age at active response to *T. parva* was 110.4 days and to *T. mutans* was 70.4 days, younger than that in the IDEAL calves.

However, a cross-sectional study of herded grazing zebu calves aged 6-18 months old found a lower prevalence of *T. mutans* compared to *T. parva* (Swai et al. 2007). Also, in a recent study in Uganda using RLB, the average age at first infection with *T. parva* was found to be 53 days, and with *T. mutans* it was found to be 74 days (Asiimwe et al. 2013), showing the opposite to IDEAL findings.

One question of interest was whether calves continued to have high levels of the species specific antibody following their seroconversion to that species. This was investigated using two methods. Figures 3.8, 3.9, 3.10, and 3.11 compare the proportion of calves that had an ELISA PP above the cut-off with the proportion of calves already in the seroconverted group. It was anticipated that the proportion of calves in both these groups should be similar if antibody levels remained high following seroconversion (after the decline of maternally acquired antibody). Figure 3.12 shows the mean PP of those calves that had already seroconverted for all four TBDs. If antibody levels were maintained following seroconversion the line would be expected to have a gradient close to zero.

For *T. parva* , *A. marginale* and *B. bigemina* figures 3.8, 3.10, and 3.11 show that the proportion of calves in the ‘seropositive’ group (over the standard cut-off) and the

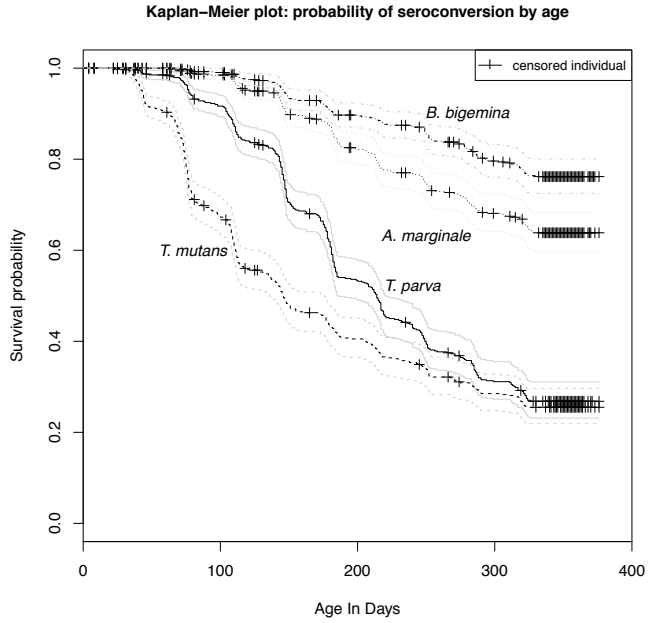

Figure 3.5: Kaplan Meier Curve for seroconversion to *Theileria parva*, *Theileria mutans*, *A. marginale* and *B. bigemina*.

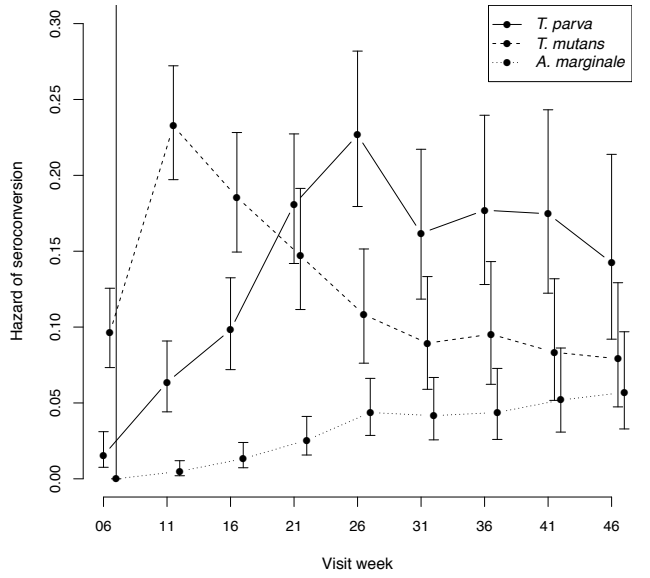

Figure 3.6: Hazard of seroconversion to *Theileria parva*, *Theileria mutans*, *A. marginale* and *B. bigemina* by visit. The vertical bars represent 95% confidence intervals.

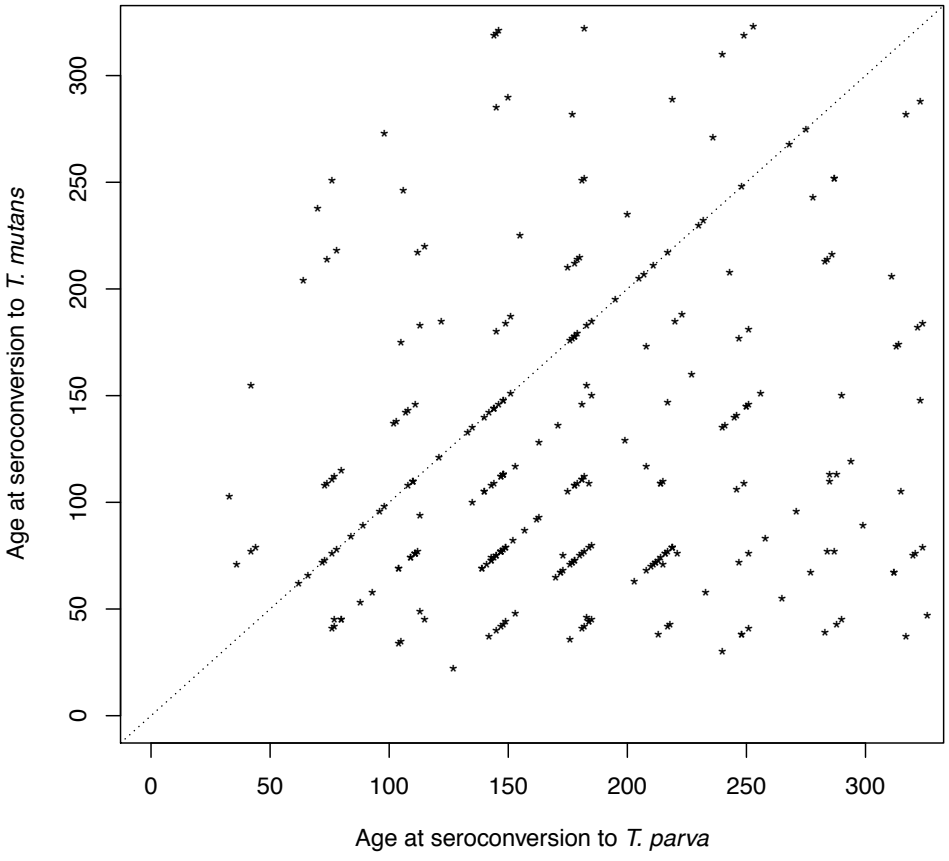

Figure 3.7: Age at seroconversion to *T.parva* plotted against age at seroconversion to *T.mutans*.

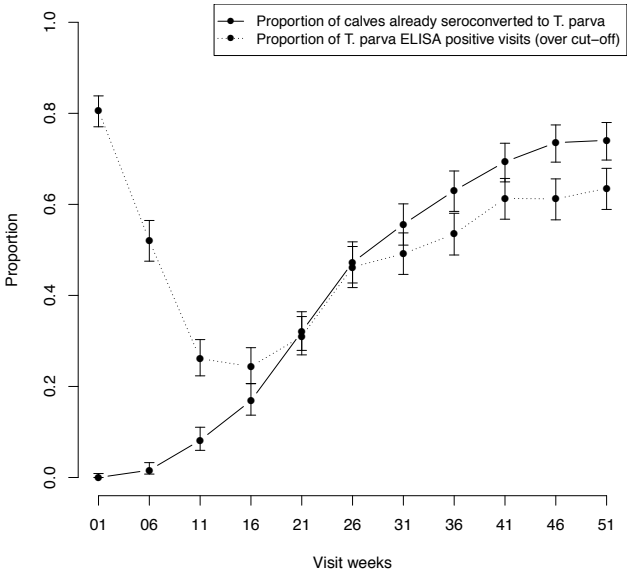

Figure 3.8: Comparison of the proportion of calves that had seroconverted to *T. parva* by week (cumulative) and the number of visits positive by ELISA for *T. parva*. The vertical bars represent 95% confidence intervals.

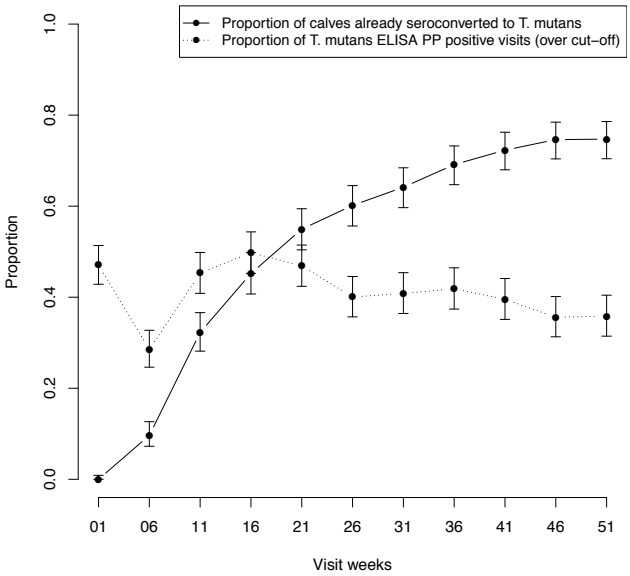

Figure 3.9: Comparison of the proportion of calves that had seroconverted to *T. mutans* by week (cumulative) and the number of visits positive by ELISA for *T. mutans*. The vertical bars represent 95% confidence intervals.

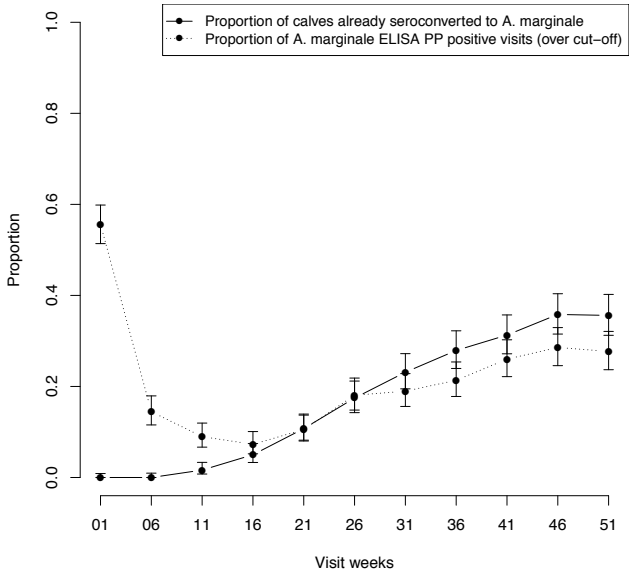

Figure 3.10: Comparison of the proportion of calves that had seroconverted to *A. marginale* by week (cumulative) and the number of visits positive by ELISA for *A. marginale*. The vertical bars represent 95% confidence intervals.

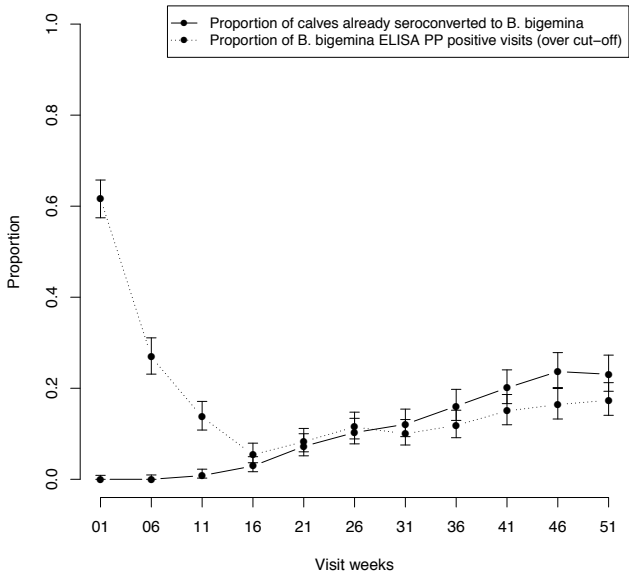

Figure 3.11: Comparison of the proportion of calves that had seroconverted to *B. bigemina* by week (cumulative) and the number of visits positive by ELISA for *B. bigemina*. The vertical bars represent 95% confidence intervals.

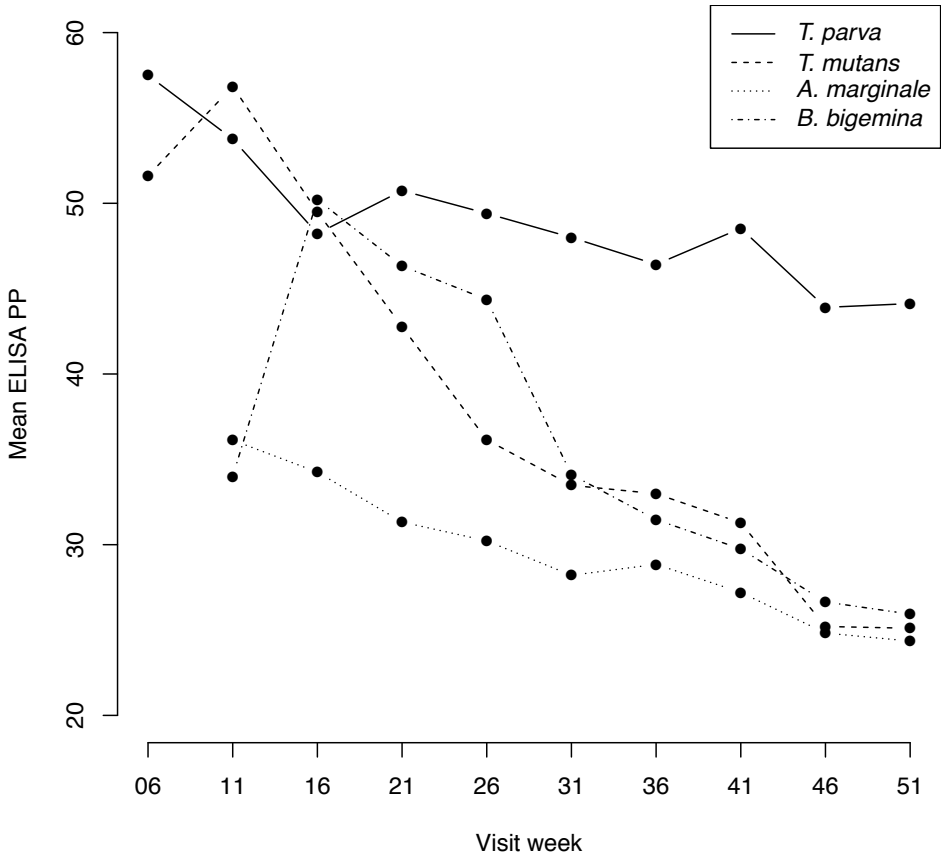

Figure 3.12: The mean ELISA PP by species. The mean is calculated from those calves that had already seroconverted by the sampling week. The vertical bars represent 95% confidence intervals.

seroconverted group were fairly similar. This effect can also be appreciated for *T. parva* and *A. marginale* from the gradual decline of the mean PP of animals following exposure (figure 3.12). This suggests that, for *T. parva* and *A. marginale*, antibody levels were sustained for some period following infection. The findings for *B. bigemina* conflict in the two plots (figures 3.11 and 3.12). The reduction in the mean PP for *T. parva* and for *A. marginale* were very similar (57% to 44% (13% difference), and 36% to 24% (12% difference) respectively).The mean PP for *A. marginale* was consistently lower than that for *T. parva* but the values remained consistently above the cut-off for all time points (>20PP for *T. parva* and >15PP for *A. marginale*).

As before, *T. mutans* showed a different trend, which nevertheless supports the findings from figure 3.3. The levels of antibody detected by the *T. mutans* ELISA declined steeply following initial exposure (figures 3.9 and 3.12). The reduction in mean PP for the seroconverted group decreased from 55% to 25% (difference of 30%). This may be because the period of higher hazard of seroconversion to *T. mutans* is concentrated across a short period when compared to *T. parva*, (figure 3.6). Therefore more of the calves represented in the later time periods were further in time from their initial exposure, whereas the calves who had already seroconverted to *T. parva* at each week were more likely to have represented a wider range of time since initial exposure. However, the data may also support the hypothesis that following initial exposure, the levels of detectable *T. mutans* antibody decline relatively quickly. Previous studies do not support this finding. This was not observed by Moll et al. (1984) following exposure of animals to *T. mutans* in a natural setting. However, indirect fluorescent antibody technique was used in this study rather than ELISA. A rapid decline was also not observed during the development of the *T. mutans* ELISA used in the IDEAL study (Katende et al. 1990). High ELISA PP values were maintained in experimentally infected animals (Friesian and Boran animals) for at least 84 days following infection, after which the experiment was terminated.

The story for *T. mutans* in this cohort from current diagnostic results is conflicting. Calves were exposed early to *T. mutans* suggesting a very high infection pressure. However, the prevalence of the infection in the dams was lower when compared to *T. parva*. Also, data suggests that antibody levels declined rapidly following first exposure conflicting with previous studies. Why, with an apparently high infection rate

in young calves, were calves not continually re-exposed leading to a constant boosting of antibody levels? Also, why in a situation where the infection pressure on young calves was apparently higher than for *T. parva* was the 1 year probability of seroconversion to *T. mutans* almost the same as for *T. parva*, and the dam prevalence lower for *T. mutans*?

The *T. mutans* antibody bound by the ELISA used in this study was against a *T. mutans* piroplasm antigen, the life-stage found in cattle erythrocytes. Katende et al. (1990) observed immune complexes at varying levels post-infection. The immune complexes were particularly common once the parasitaemia had reduced to low levels. If this phenomenon had occurred in the samples tested, the antibodies available for binding to the conjugate in the ELISA would have been fewer leading to a lower sensitivity for the test. If this phenomenon had occurred, it is likely that seroconversion following exposure would have been observed at the appropriate time at a population level, but possibly in fewer calves. It may also have caused the levels of antibody detected by the ELISA to have declined as immune complexes became more common.

An alternative hypothesis is that the anti-bovine antibody isotope used in the conjugate was too specific. The conjugate used only recognises the IgG_1 isotype (personal communication: Philip Toye, ILRI). The test was designed this way to improve its specificity. The predominant IgG isotype taken up into the lacteals during colostrum production is IgG_1 at a ratio of about 7:1 when compared to IgG_2 (Sasaki et al. 1977). Therefore, the ELISA is very well suited for detection of maternally derived antibody in calves. It is also evident from our results that calves exposed to *T. mutans* did generate adequate quantities of *T. mutans* specific IgG_1 for seroconversion to be detected following infection. However, higher levels of IgG_2 compared to IgG_1 at any point during the post-exposure immune response would have reduced the sensitivity of the test. This is because, in the case of more numerous IgG_2, it would have had the potential to occupy the majority of antibody binding sites on the ELISA plate. As this isotype was not recognised by the conjugate used in the test the bound IgG_2 antibody would not have led to a colour change.

The conflicting results from *T. mutans* hint at a lowered sensitivity for the *T. mutans* ELISA, but further work would need to be done to fully investigate this, including

analysis of immune complexes and the IgG isotypes produced following natural infection with *T. mutans*.

3.3.4 The proportion of calves exposed and the mean age of seroconversion by sublocation

The random selection of sublocations was stratified by agroecological zone to capture as much geographical variation as possible across the study site. Tick distribution is affected by local climatic conditions and vegetation types and cover, and agroecological zone is a way of stratifying areas with similar topographical features and suitability for different crops. This may have correlated with suitability for ticks, and so may have led to differences in risk of exposure to the tick borne disease across the study site.

Maps of the mean age at seroconversion and the proportions of animals exposed in each sublocation across the study site allowed trends to be investigated. Figures 3.13, 3.14, and 3.15 show the mean age at seroconversion by sublocation. The grey regions distinguish the different AEZs. There was not a strong geographical pattern of age at seroconversion for any of the species shown here. There was also not a strong geographical pattern in the proportion of calves that seroconverted by one year (figures 3.16, 3.17, and 3.18). When proportions and mean age by sublocations were compared between species no correlation was observed (e.g those sublocations with a high mean age of seroconversion for *T. mutans* did not also have a higher mean age for *T. parva*). There is little evidence here for spatial variation in the infection pressure of *T. parva* or *T. mutans*.

However, when plotting the by week hazard of seroconversion to *T. parva* by AEZ, and elevation it can be seen that there was an increased hazard of seroconversion for calves that were in homesteads in the lowest quartile of elevation ($<$1199m), and that there was an increased hazard of seroconversion for calves from AEZ 5. This was the AEZ nearest to Lake Victoria (figures 3.19 and 3.20). Therefore, although figures 3.14 and 3.17 do not show a strong geographical trend, there is evidence for some difference according to location.

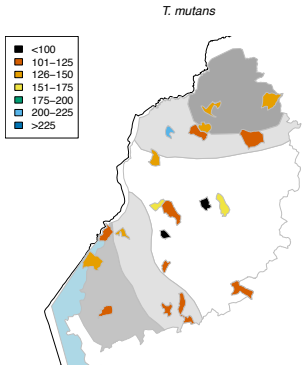

Figure 3.13: The mean age (days) of seroconversion to *T. mutans* by sublocation.

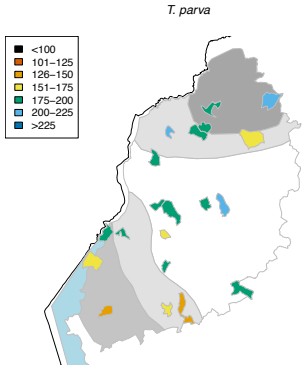

Figure 3.14: The mean age (days) of seroconversion to *T. parva* by sublocation.

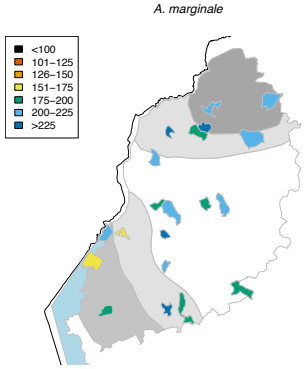

Figure 3.15: The mean age (days) of seroconversion to *A. marginale* by sublocation.

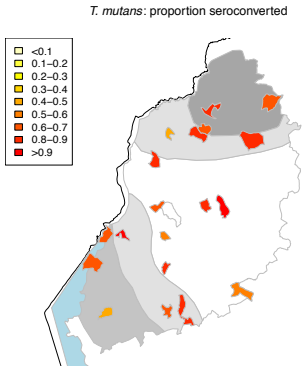

Figure 3.16: The proportion of those within each sublocation that seroconverted to *T. mutans* by one year old.

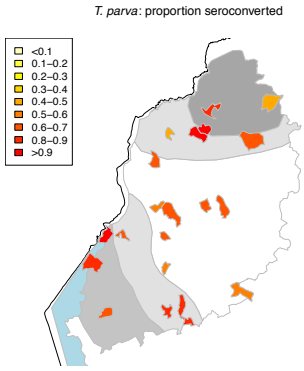

Figure 3.17: The proportion of those within each sublocation that seroconverted to *T. parva* by one year old.

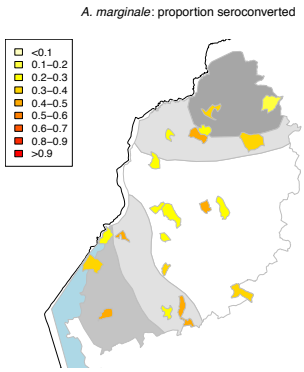

Figure 3.18: The proportion of those within each sublocation that seroconverted to *A. marginale* by one year old.

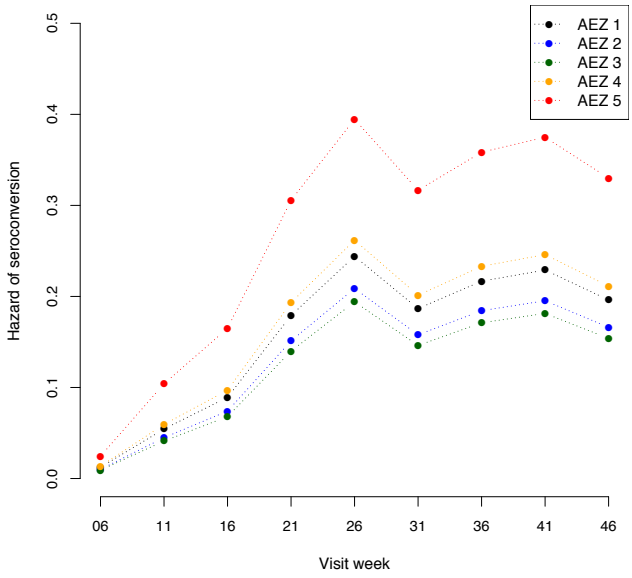

Figure 3.19: The hazard of seroconversion to *T. parva* by AEZ.

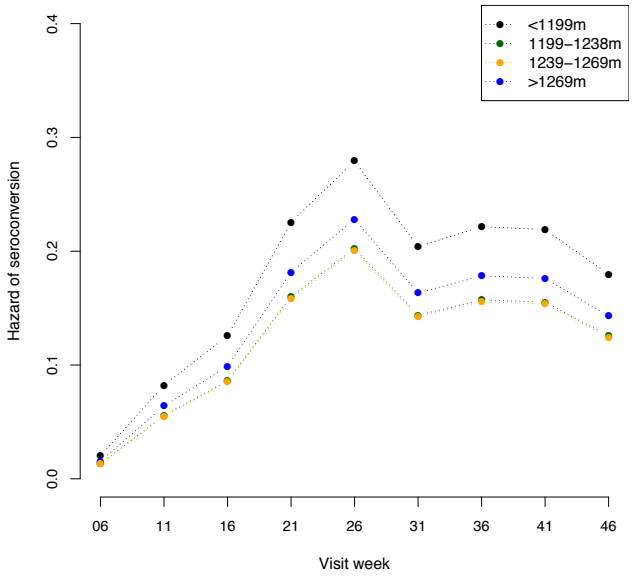

Figure 3.20: The hazard of seroconversion to *T. parva* by elevation.

3.3.5 The tick vectors

Table 3.4 summarises the tick vector associated with each of the species of *Theileria*. It can be seen from this that the vectors remain unidentified for several species of *Theileria*.

Table 3.4: *Theileria* species and their tick vectors (Norval et al. 1992).

Theileria spp.	Tick Vector
T. parva	*Rhipicephalus appendiculatus*
T. mutans	*Amblyomma variegatum*
T. taurotragi	*Rhipicephalus appendiculatus*
T. velifera	*Amblyomma variegatum*
T. sable	Unknown
T. ovis	Unknown
T. bicornis	Unknown

The mean age at first infection with *Amblyomma variegatum* was 138.8 days and that of *Rhipicephalus appendiculatus* 19.1 days. The mean proportion of visits positive for *R. appendiculatus* per calf was 0.9. The mean proportion of visits positive for *A .variegatum* was 0.42. The difference in age at first infestation between the two species of tick that transmit *T. parva* and *T. mutans* is clearly shown in figure 3.21. Many of the calves were already infested with *R. appendiculatus* at the recruitment visit. The very early infestation with the vector for *T. parva* and the later infestation with the vector for *T. mutans* contradicts with the earlier finding that the mean age of exposure to *T. mutans* was lower than for *T. parva*.

Figure 3.22 compares the age at seroconversion to *T. parva* and *T. mutans* with the age at first infestation with the two tick vectors. It can be seen that there is little correlation between first infestations with *R. appendiculatus* and seroconversion to *T. parva*, apart from tick infestation always preceding seroconversion (as would be expected). The infestation of very young calves, those calves that were unlikely to have left the homestead, may be explained by the diurnal habits of *R. appendiculatus*. This tick commonly detaches from its hosts in the early morning before the adults leave for grazing, leading to an infestation of the homestead (Norval et al. 1992).

The surprising results relate to *A. variegatum*. A total of 179 calves seroconverted to *T. mutans* before the vector of *T. mutans*, *A. variegatum*, was recorded on the calf (figure

3.22). It could be that *T. mutans* was transmitted by a species of tick other than *A. variegatum*. *R. appendiculatus* would be a good candidate due to the early age of infestation. It can also be seen in figure 3.22 that *R. appendiculatus* infestation always preceded *T. mutans* seroconversion. However, during experimental investigation of the transmission of *T. mutans* by proposed vectors, *R. appendiculatus* did not transmit this species (Young et al. 1978). It is more likely that the ticks transmitting *T. mutans* were not observed. Both *R. appendiculatus* and *A. variegatum* are three host ticks. They are able to transmit the infection as nymphs. However, the IDEAL project only recorded adult stage ticks. It is possible that the very small *A. variegatum* nymphs were not recorded, but were responsible for significant amounts of transmission of *T. mutans*. Moll et al. (1984) found a higher *Theileria* infection rate in *A. variegatum* than in *R. appendiculatus*. In a future study, it would be advisable to investigate the numbers of different tick life-stages on calves, and to systematically collect *A. variegatum* nymphs and adults from within the study site and to measure the comparative number of infected ticks and infected salivary glands.

3.3.6 Reverse line blot testing on final visits

The reverse line blot hybridisation test (RLB) was used to identify the haemoparasite infections present in the blood taken at the final routine visit to calves. 455 calves survived to one year. The samples from 3 calves were not tested although they reached their final visit at one year old. This was due to an error in the laboratory.

The proportions of calves found to be positive for each of the *Theileria* species are summarised in table 3.5. Figure 3.23 shows the number of different *Theileria* found in each of the calves at their final visits. 88 of those calves surviving to one year old had no *Theileria* species identified by RLB at their final visit (19%). The maximum number of species found in a single calf was 5.

MCA was applied to the RLB data to investigate whether certain *Theileria* species tended to co-occur within calves.

Dimension 1 explained 28.3% of the variation between individuals. The presence of *T. parva*, *T. taurotragi*, and *T. bicornis* acted positively along this axis, and the presence

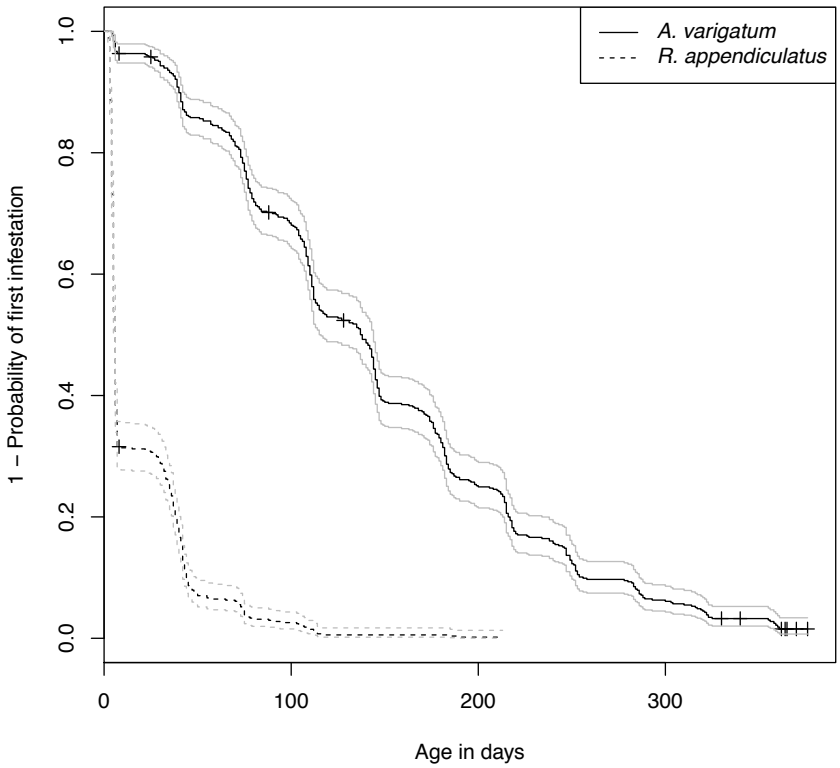

Figure 3.21: Kaplan Meier curve for age to first infestation with *R. appendiculatus* and *A. variagtum*.

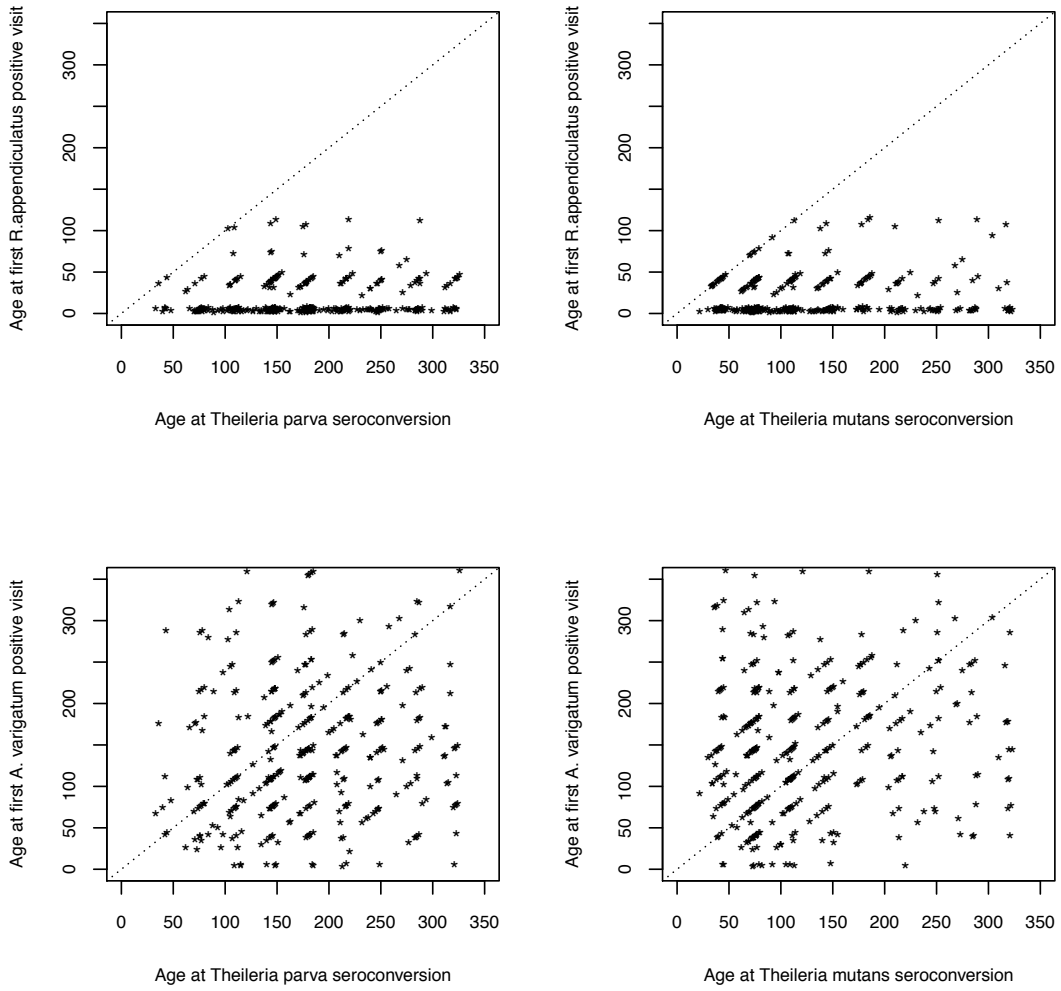

Figure 3.22: Age in days at first infestation with ticks compared to age at *Theileria parva* and *Theileria mutans* seroconversion.

Table 3.5: Summary of results for RLB testing at the final visit for those calves that survived to one year.

Species	Neg.	Pos.	Propn.
T. parva	397	55	0.12
T. taurotragi	419	33	0.07
T. mutans	140	312	0.69
T. velifera	167	285	0.63
T. spp (sable)	315	137	0.30
T. bicornis	446	6	0.01
T. ovis	438	14	0.03

of *T. ovis*, *T. mutans*, *T. spp* (sable), and *T. velifera* acted negatively along this axis (figure 3.24).

Dimension 2 explained 22.95% of the variation. The presence of *T. parva*, *T. taurotragi*, and *T. bicornis* acted positively on this axis, and the absence of all species acted negatively.

Following the exclusion of the rare species (*T. bicornis* and *T. ovis*, figure 3.25) dimension 1 explained 36.9% of the variation and dimension 2, 27.49% of the variation. Dimension 1 had very similar qualities to the previous analysis including all species. For dimension 2 the presence of *T. parva*, *T. mutans*, and *T. taurotragi* acted positively, and their absence acted negatively on the axis. The plot of dimension 1 against dimension 2 (figure 3.24) suggests that there were two sets of species that tended to co-occur within individuals.

The inter-relationship between species was further investigated using logistic regression. Models were built where the presence of one species was predicted by the presence of the others. Too few cases of *T. bicornis* and *T. ovis* were identified to investigate these as outcomes. Table 3.6 summarises the models for the prediction of the presence of each of the *Theileria* species.

Identification of *T. mutans* and, *T. taurotragi* by RLB significantly increased the odds of identifying *T. parva*.

T. velifera significantly reduced the odds of finding *T. parva*.

Identification of *T. parva*, *T. taurotragi*, *T. velifera*, and *T. sable* significantly increased the odds of identifying *T. mutans*.

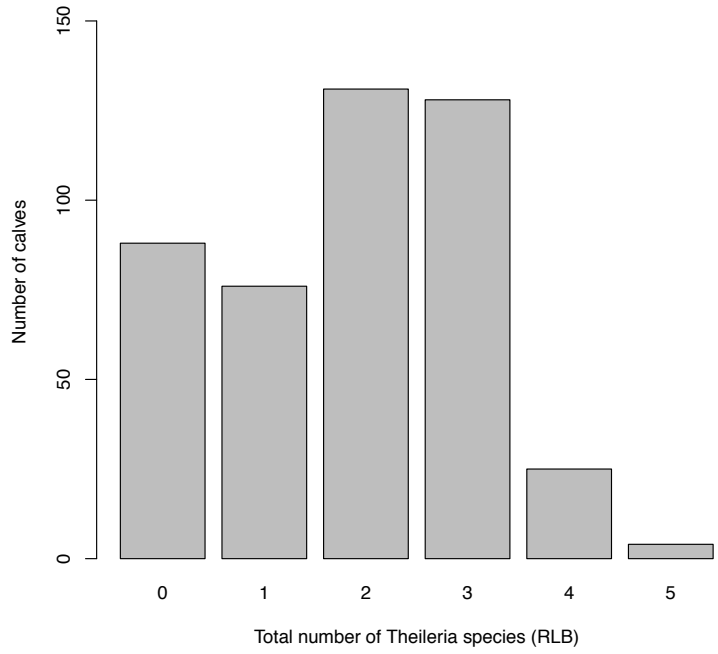

Figure 3.23: The total number of *Theilieria* species found by RLB in each calf.

Identification of *T. parva*, and *T. mutans* significantly increased the odds of identifying *T. taurotragi*.

T. velifera significantly reduced the odds of finding *T. taurotragi*.

Identification of *T. mutans* significantly increased the odds of identifying *T. velifera*.

T. parva and *T. taurotragi* significantly reduced the odds of finding *T. velifera*.

Identification of *T. mutans* significantly increased the odds of identifying *T. spp* (sable).

T. parva significantly reduced the odds of finding *T. spp* (sable).

The relationships identified by the models can be represented as shown in figure 3.26. This figure resembles the findings of MCA (figure 3.24).

T. mutans and *T. velifera* share the same tick vector (*A. variegatum*) as do *T. taurotragi* and *T. parva* (*R. appendiculatus*) (table 3.4), and this may explain why these two pairs

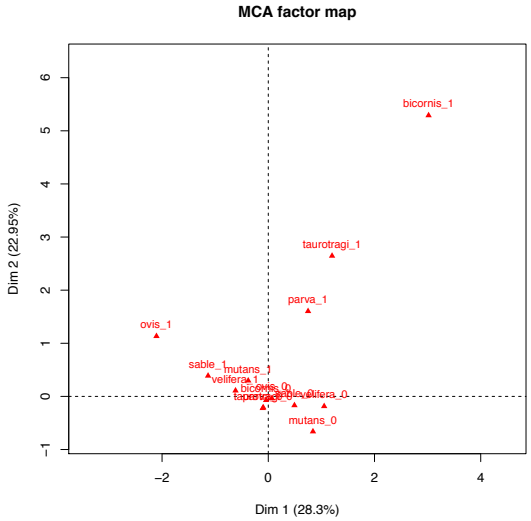

Figure 3.24: Plot of the first two dimensions for MCA investigating the co-occurrence of species of *Theileria* within calves.

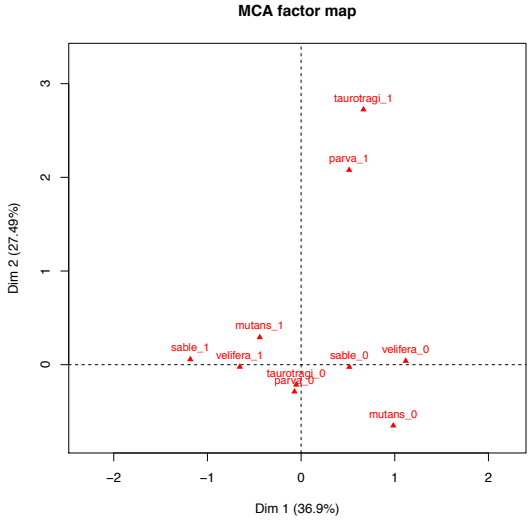

Figure 3.25: Plot of the first two dimensions for MCA investigating the co-occurrence of species of *Theileria* within calves. The rare species (*T. bicornis* and *T. ovis*) were excluded from this analysis.

of species were found. However, *T. mutans* was associated with increased odds of finding *T. parva* and *T. taurotragi* although it does not share a vector with them. Both *T. parva* and *T. mutans* were very common in the cohort, and this may explain their common co-occurrence at one year old. However, *T. velifera* was also very common and there is not evidence for co-occurrence of *T. parva* and *T. velifera*. However, no shared risk factors for hazard of seroconversion to *T. mutans* and *T. parva* were identified (see section 3.3.9 in this chapter). The reasons for the interconnection between these species remain unresolved.

It has previously been reported that the most likely vector of *Theileria* spp. (sable) is *R. appendiculatus* (Nijhof et al. 2005). However, the relationships between species in this study suggest that the vector may be shared with *T. mutans* and could be *A. variegatum* as for *T. velifera*.

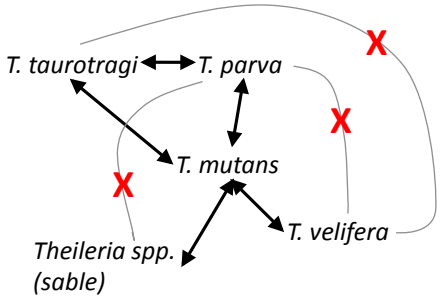

Figure 3.26: Diagram showing relationships between *Theileria* species according to logistic regression.

3.3.7 Comparing findings from different diagnostic tests

The findings of the diagnostic tests for *T. mutans* and *T. parva* were compared for samples from the final visits at one year. Pairwise comparisons were made using 2 by 2 tables, and Cohen's Kappa measurement of agreement. The comparisons are in tables 3.7, 3.8, and 3.9.

Generally, the agreement between tests was poor. This was not unexpected, especially

Table 3.6: Summary of logistic regression models for the prediction of the presence of one *Theiieria* species within a calf by other *Theileria* species.

T. parva	Estimate	SE	Pvalue	OR	LCL_OR	UCL_OR
(Intercept)	-2.571	0.361	<0.001	0.076	0.035	0.146
T. mutans +ve	1.140	0.418	0.006	3.127	1.432	7.474
T. taurotragi +ve	1.837	0.415	<0.001	6.279	2.758	14.167
T. velifera +ve	-0.881	0.336	0.009	0.414	0.214	0.804
T. mutans	Estimate	SE	Pvalue	OR	LCL_OR	UCL_OR
(Intercept)	-0.588	0.178	0.001	0.555	0.390	0.783
T. parva +ve	1.186	0.428	0.006	3.275	1.466	7.976
T. taurotragi +ve	1.197	0.556	0.031	3.311	1.183	10.857
T. velifera +ve	1.723	0.263	<0.001	5.604	3.378	9.494
T. spp (sable) +ve	1.021	0.354	0.004	2.776	1.417	5.731
T. taurotragi	Estimate	SE	Pvalue	OR	LCL_OR	UCL_OR
(Intercept)	-3.260	0.479	<0.001	0.038	0.013	0.089
T. parva +ve	1.826	0.412	<0.001	6.207	2.745	13.925
T. mutans +ve	1.271	0.543	0.019	3.566	1.313	11.475
T. velifera +ve	-1.364	0.419	0.001	0.256	0.110	0.576
T. velifera	Estimate	SE	Pvalue	OR	LCL_OR	UCL_OR
(Intercept)	-0.634	0.182	<0.001	0.531	0.369	0.753
T. parva +ve	-0.946	0.338	0.005	0.388	0.200	0.756
T. mutans +ve	2.158	0.238	<0.001	8.655	5.468	13.939
T. taurotragi +ve	-1.422	0.419	0.001	0.241	0.104	0.545
T. sable	Estimate	SE	Pvalue	OR	LCL_OR	UCL_OR
(Intercept)	-2.234	0.292	<0.001	0.107	0.058	0.182
Tparva +ve	-1.041	0.374	0.005	0.353	0.161	0.709
Tmutans +ve	1.951	0.316	<0.001	7.039	3.921	13.633

as each of the tests measured a slightly different outcome. Seroconversion measured whether the calf had been exposed at any time during the previous year. A single ELISA result measured a current antibody response to a current or previous infection, and RLB and microscopy measured the presence of parasite in the sample.

For *T. parva*, there were many animals that were positive by ELISA, or seroconversion, but were negative by RLB. However, this is not surprising because being seropositive does not necessarily denote a current infection, and seroconversion identified any exposure through the previous year.

For *T. mutans*, there were many animals that were positive by RLB, but negative by ELISA, the opposite to results observed for *T. parva*. This is surprising as it suggests that there were a lot of new infections detected at the final visit that the calf had not yet mounted an antibody response to. However, figure 3.6 showed the peak hazard of infection with *T. mutans* was at a relatively young age, and it can be seen that a lot of animals that had seroconverted previously were RLB positive (table 3.8). These infections were either due to persistent or re-infection following the initial infection. This finding offers further support for the hypothesis discussed earlier, that following initial infection, the ELISA was no longer able to detect antibody to *T. mutans* even though the parasite persisted in the host.

RLB and MCR were most likely to be correlated as they both identified actual parasite. Neither of the tests used in this context were species specific. A calf was identified as RLB *Theileria* species positive if at least one *Theileria* species was identified by the RLB. The majority of animals were positive by both tests (table 3.9). However, more calves were positive by RLB and negative by microscopy than were negative by RLB and positive by microscopy. This is most likely due to the higher sensitivity of PCR based tests compared to microscopy.

Calves were not frequently detected as carriers of *T. parva*. Only 45 calves that had seroconverted to *T. parva* in their first year had *T. parva* detected by RLB at their final visit, as opposed to 292 that were negative for *T. parva*. The carrier state is believed to be very important for maintaining levels of infection in *R. appendiculatus*, and is a component of the description of how endemic stability is maintained for *T. parva* (Norval et al. 1992). However, it may be that although the carrier state was rare in

calves, it may be more common in adult animals. It is also possible that RLB is not an optimum method of identifying the carrier state, due to a possible low sensitivity. However, the presence of piroplasms in blood would be essential for transmission to ticks, and the use of material from lymph nodes would not be appropriate for the assessment of the chance of onward transmission.

3.3.8 Diagnosis of *Theileria parva* at post-mortem

There were 34 cases of East Coast Fever recorded in the cohort. In 32 of these cases ECF was defined as the primary cause. In two it was the secondary cause to black quarter (*Clostridia* infection) or heartwater (*Ehrlichia ruminatum*). Of the 34, 28 calves were first detected as being infected with *T. parva* at their post-mortem examination. The risks associated with death due to *T. parva* are investigated in chapter 6.

3.3.9 Risk factors associated with seroconversion

Time discrete hazard analysis was used to investigate the exposures associated with hazard of seroconversion to *T. parva* and *T. mutans*. The method provides a hazard ratio for exposures (chapter 2, section 2.4.4). Both time dependent and time independent exposures were investigated. A list of all those variables screened for association in univariable models is in table H.1 in the appendix. Model selection was carried out as described in chapter 2. section 2.4.5.

Exposures associated with the hazard of seroconversion to *T. parva*

Table 3.10 summarises the final model for hazard of seroconversion to *T. parva*. A random effect for sublocation did not improve the fit of this model so was not included. More animals (tropical livestock units) and more applications of the antiobiotic, oxytetracycline, were associated with an increased hazard of seroconversion, as was whether the calf was infected with the tick, *A. variegatum* at the visit of seroconversion. Homesteads at higher elevations had a decreased hazard of seroconversion.

Table 3.7: Comparison of serological based testing and RLB for *T. parva* for those calves reaching their final visit

	RLB_parva_neg	RLB_parva_pos
ELISA_parva_neg	150	13
ELISA_parva_pos	247	42
Cohen's Kappa	0.049	
	RLB_parva_neg	RLB_parva_pos
SC_parva_neg	105	10
SC_parva_pos	292	45
Cohen's Kappa	0.026	

Table 3.8: Comparison of serological based testing and RLB for *T. mutans* for those calves reaching their final visit

	RLB_mutans_neg	RLB_mutans_pos
ELISA_mutans_neg	85	204
ELISA_mutans_pos	55	108
Cohen's Kappa	-0.036	
	RLB_mutans_neg	RLB_mutans_pos
SC_mutans_neg	39	75
SC_mutans_pos	101	237
Cohen's Kappa	0.04	

Table 3.9: Comparison of result of microscopy for *Theileria* spp. and being positive for any *Theilieria* species by RLB at routine visit week 51.

	RLB_neg	RLB_pos
MCR_neg	21	97
MCR_pos	67	267
Cohen's Kappa	-0.025	

Many of the calves remained in the homestead while the herd went out grazing during the day, especially early in life. An increase in tropical livestock units suggests a larger number of animals were herded from the homestead each day and back at night. The vector of *T. parva*, *R. appendiculatus* tends to drop from its host early around dawn. Therefore, calves from homesteads with a large number of animals may have been exposed to an increased number of ticks that had dropped from their more numerous herd mates, in turn increasing their chance of infection. However, it was the presence of *A. variegatum* that was associated with an increased hazard of seroconversion to *T. parva* rather than the presence of *R. appendiculatus*. *R. appendiculatus* was very common and animals had very few visits free from *R. appendiculatus*. However, only a small percentage of *R. appendiculatus* are infected with *T. parva* at a time, so each bite has a low probability of transmitting infection. Simple presence or absence of *R. appendiculatus* was not associated with seroconversion to *T. parva*. Analysis showed that *A. variegatum* ticks were less common than *R. appendiculatus*. It could be that *A. variegatum* acted as a sentinel for larger infestations with *R. appendiculatus*. This of course relies on the assumption of co-distribution of *R. appendiculatus* and *A. variegatum*.

Higher elevations were associated with a decrease in the hazard of seroconversion. Higher elevations are usually correlated with a drop in temperature, but in the IDEAL study site, higher elevations were also correlated with increasingly rocky terrain. It is likely that areas at higher elevations were less suitable habitats for ticks, and this resulted in a decrease in infection pressure.

Antibiotics are used for the treatment of many different infections. However, one widely used antibiotic, oxytetracycline is used particularly for the treatment of early stage ECF cases. Antibiotic use may have acted as a proxy for ECF cases in the herd and so a proxy for higher local infection pressures of *T. parva*. This would have led to an increased hazard of seroconversion for calves in these herds.

Exposures associated with the hazard of seroconversion to *T. mutans*

Table 3.11 summarises the parsimonious model for hazard of seroconversion to *T. mutans*. Only two exposures were significantly associated with the hazard of

Table 3.10: TDHA model for *T. parva*. Sublocation as a random effect did not improve model fit so was not included in the model. Calf as a random effect was included (variance = $7.34\text{x}10^{-11}$, standard deviation = $8.57\text{x}10^{-6}$).

	Week	Estimate	SE	P value	Hazard	CI 2.5% haz	CI 97% haz
	06	-4.690	0.402	<0.001	0.009	0.004	0.020
	11	-3.239	0.261	<0.001	0.038	0.023	0.061
	16	-2.775	0.242	<0.001	0.059	0.037	0.091
	21	-2.095	0.223	<0.001	0.110	0.074	0.160
	26	-1.822	0.224	<0.001	0.139	0.094	0.201
	31	-2.259	0.254	<0.001	0.095	0.060	0.146
	36	-2.173	0.261	<0.001	0.102	0.064	0.160
	41	-2.202	0.276	<0.001	0.100	0.060	0.160
	46	-2.438	0.307	<0.001	0.080	0.046	0.137
	Exposures						
log(Tropical livestock units)		0.407	0.094	<0.001	0.600	0.555	0.644
Elevation <1199m		-	-	-	-	-	-
Elevation 1199-1238 m		-0.190	0.163	0.244	0.453	0.375	0.532
Elevation 1239-1269 m		-0.232	0.163	0.155	0.442	0.365	0.522
Elevation >1269 m		-0.406	0.168	0.016	0.400	0.324	0.481
No. antibiotics treatment		0.111	0.048	0.021	0.528	0.504	0.551
Calf visit *A. variegatum* -ve		-	-	-	-	-	-
Calf visit *A. variegatum* +ve		0.294	0.122	0.016	0.573	0.514	0.630

seroconversion to *T. mutans*. A decrease in hazard of seroconversion to *T. mutans* was associated with a high *T. mutans* ELISA antibody titre at birth. An increase in hazard of seroconversion to *T. mutans* was associated with the presence of *R. appendiculatus* at the visit of seroconversion.

Little is known about the bovine immune response to *T. mutans*. No published work was found about the role of maternal antibody and *T. mutans* infection in offspring. It has been shown that calves can mount a humoral immune response to infection very early in life in the absence of maternal antibody (Hodgins and Shewen 2000), and that the presence of maternal antibody may reduce or stop the antibody response to a vaccine given to two and four weeks old calves (Hodgins and Shewen 1998). Therefore, it is possible that those calves with higher levels of maternal antibody to *T. mutans* were less able to mount an immune response following natural exposure. However, it may also be that the seroconversion rule was not able to detect seroconversion in the presence of high levels of maternal antibody.

The finding that *R. appendiculatus* was associated with an increased hazard of

seroconversion to *T. mutans* seems particularly odd, especially considering the lack of association between *R. appendiculatus* and seroconversion to *T. parva*. *T. parva* seroconversions occurred comparatively late. However, seroconversion to *T. mutans* occurred earlier in life, when there were still a number of animals not infested with *R. appendiculatus*. This raises the question whether *R. appendiculatus* ticks were acting as a sentinel for early infection with *A. variegatum*, particularly the nymphs that were not recorded during this study.

Table 3.11: TDHA model for *T. mutans*. A random effect for calf was included (variance = $9.8\text{x}10^{-11}$, SD = $9.9\text{x}10^{-6}$

Week	Estimate	SE	Pvalue	Hazard ratio	CI2.5_haz	CI97.5_haz
06	-2.686	0.311	<0.001	0.064	0.036	0.111
11	-1.631	0.289	<0.001	0.164	0.100	0.257
16	-1.935	0.305	<0.001	0.126	0.074	0.208
21	-2.225	0.323	<0.001	0.098	0.054	0.169
26	-2.573	0.342	<0.001	0.071	0.038	0.130
31	-2.771	0.360	<0.001	0.059	0.030	0.113
36	-2.703	0.366	<0.001	0.063	0.032	0.121
41	-2.867	0.384	<0.001	0.054	0.026	0.108
46	-2.925	0.396	<0.001	0.051	0.024	0.105
Exposures						
T. mutans PP at recruitment	-0.010	0.003	0.001	0.497	0.496	0.499
Calf visit *R. appendiculatus* -ve	-	-	-	-	-	-
Calf visit *R. appendiculatus* +ve	0.748	0.279	0.007	0.679	0.550	0.785

3.4 Summary of findings and suggested further work

One of the major, but perhaps least surprising finding of this work was how common *Theilieria* species were in the study cohort. 66% of calves were detected as seroconverting to *T. parva* and 69% to *T. mutans*. *Theileria* spp. were identified in blood smears in the majority of visits. 81% of calves had maternally derived antibodies against *T. parva* and 47% against *T. mutans*. Species of *Theileria* were more likely to be found together within a host if they shared the same tick vector. Calves were infected with *R. appendiculatus* from a very young age, but the first exposure to *T. mutans* tended to occur before that to *T. parva*.

There was no pattern in the variation in infection pressure according to geographical location, and sublocation did not significantly contribute to models of hazard of seroconversion. However, lower elevation homesteads were significantly associated with an increase in the hazard of seroconversion to *T. parva* in a multivariable model. These lower elevation homesteads were generally found closer to Lake Victoria, often in AEZ 5.

Data collected by this study provided the opportunity to start to describe the relationship between *T. parva* and *T. mutans* and their vectors at a population and host level. However, the comparison presented contradictory results, and raised questions about the antibody response following *T. mutans* infection, and the relationship between actual circulating antibody levels and those that were detected by the ELISA.

It would be very useful to expand on the data currently available for analysis. More detail is needed to strengthen some of the findings, and to attempt to resolve some of the questions posed. Whole blood and serum samples from all routine visits are available. The costs and time investment involved in further testing will be a major limiting factor. However, the testing of every visit by RLB would allow a more detailed examination of which species of *Theileria* and other haemoparasite species were present in which calves and when. More frequent sampling may have led to a better understanding of the decay of maternal antibody and the levels of antibody following infection. However, the priority should be to investigate both the presence of immune complexes and the IgG isotypes of *T. mutans* antibody present in samples at different times following initial infection.

Further investigation of tick dynamics should concentrate on the infestation levels of the different life-stages on calves, and the infection prevalence and intensity in ticks in the region. No material from ticks was collected during data collection so this will not be able to be linked to cohort calves.

Chapter 4

Expression of infectious disease in the cohort

Chapter abstract

This chapter describes the clinical signs observed in the cohort calves, especially those associated with mortality. The aims were to describe clinical syndromes in the cohort and more specifically, to describe a distinct clinical syndrome expressed by the IDEAL calves in association with ECF, to investigate how these syndromes may have varied from that typically described in text books, and to investigate the possibility of the use of decision support tools in this population. Examination of quantitative and qualitative clinical signs identified signs commonly observed with ECF in the cohort. However, there was a large amount of clinical variation between calves that died of the same definitive cause. This is believed to be both because clinical cases were observed at different stages in disease progression, and because of the frequent co-infections which complicated clinical presentations. A decision support tool was found to have potential for the diagnoses of ECF, but it is suggested that it would need modifications for use in this setting.

4.1 Introduction

Infectious disease is associated with loss of productivity in livestock. Many tools are available for the diagnosis of infection. However, the identification of an infection, or exposure to an infection is not synonymous with the detection of disease. The diagnosis of disease requires the identification of a set of clinical signs (a syndrome) characteristic of a particular infection in addition to the identification of the infectious agent. However, access to diagnostic tests is limited due to cost or access, and this is especially the case in isolated rural areas such as Western Kenya where access to veterinary services is limited. Many diagnoses are made on a clinical examination alone through necessity. Prior knowledge of the local risks, and patient signalment may allow a diagnosis to be made. However, little known about how accurate the clinical diagnoses made at the pen side are. These diagnoses will also be further confused because animals are often co-infected with several potential pathogens at once. Accurate case definition is not only essential for the treatment of the individual animal, but syndromic surveillance is also important in assessing the impact of a current infection in a population, and through constant monitoring of clinical signs and their relationship to syndromes is can allow the identification of new emerging clusters of clinical signs that may give evidence for the presence of a new disease (Dórea et al. 2011). It is necessary to form case definitions that are effective in a field situation. Such case definitions have been exploited by some authors to attempt to aid field veterinarians to diagnose and treat cases of disease without the use of further laboratory diagnostic techniques (Eisler et al. 2007; Mckendrick et al. 2000; Sergeant 2009).

A prototype card-based clinical decision support tool (DST) was developed for use with cattle in sub-Saharan east Africa by Eisler et al. (2007). The tool was designed to be used at the pen-side to aid in the diagnoses and treatment decisions made by technical animal health staff. The tool was developed from data collected by Delphi survey. The surveys were completed by experts in tropical cattle diseases. The eight diseases included in the tool were those identified by the author as having significant impact on cattle production in the target region. The surveys collected the clinical signs that the experts associated with these eight diseases. The surveys were conducted in two rounds. At the second round the experts knew the opinions of their peers from

the first round. Once the survey data were collected, signs that were reported by fewer than 1% of respondents were excluded, and all remaining signs were examined for clustering between the diseases of interest. Each sign was then weighted for its association with the different diseases, and given a number of points for each disease. More points indicates that that sign is more strongly associated with a disease. When using the card, the operative counts up the number of points collected for each disease on the card dependent on the clinical signs present. Between 15 and 16 points are available for every disease. The clinical sign points are colour coded to identify the size of the 'effect' of that sign on the diagnosis of a disease. The card is shown in figure 4.1. The tool decides on the most likely diagnosis by comparing scores between the possible causes. The disease with the highest score is defined as the most likely diagnosis. Some testing was carried out investigating how experience of the tool changed the behaviour of field based veterinary professionals and para-professionals (Eisler et al. 2012). However, there has not been a quantitative assessment of the sensitivity or specificity of the diagnosis of the tool for specific diseases, or how often an individual animal is correctly diagnosed using the tool.

CaDDiS in an online diagnostic support tool that employs a bayesian belief network to predict the most likely diagnosis following the input of clinical signs by farmers (Mckendrick et al. 2000). It is a web based platform and used expert opinion to define clinical syndromes as for the decision support tool described by Eisler et al. (2007). The surveyed clinicians were asked to estimate the prevalence of particular clinical signs for certain diseases. CaDDiS concentrates on 20 diseases important in the tropics, and uses 27 clinical signs . It therefore has more breadth compared to the card based support tool described in Eisler et al. (2007). It uses information on the probability of the cow suffering from disease A dependent on clinical sign B being present, and the probability of clinical sign B being present given that the cow has disease A. The web based platform is no longer available on line.

AusVet (http://www.ausvet.com.au/), in partnership with the Australian government, developed a web based syndromic surveillance system, BoSSS (Bovine Syndromic Surveillance System), to help farmers to gain information and aid in the diagnoses of diseases in their cattle, and simultaneously the system collected data on behalf of the government to monitor endemic diseases and provide and early warning of exotic

	Anaplasm.	Babesiosis	Cowdriosis	Fasciolosis	PGE	Schistosm.	Theileriosis	Trypanosm.
Anaemia or Pallor	4	2		2	3	4	1	4
Anorexia or Depression	2	2	4				3	
Ataxia or Abnormal behaviour			4					
Constipation	4							
Diarrhoea				1	3	1		
Dysentery						2	1	
Dyspnoea or Coughing							3	
Haemoglobinuria		4						
Icterus	1	2						
Lymph node enlargement							4	2
Pyrexia	3	4	4				4	1
Staring coat				2	2	1		3
Stunted growth or pot belly				2	3	2		
Submandibular/ventral oedema				3	2			
Weakness	1	2	3	3	1	3		2
Weight loss	1			3	2	3		4

Figure 4.1: Prototype decision support card (Eisler et al. 2007). ©Cambridge University Press 2006

diseases in the national herd (Baldock et al. 2006). The programme encouraged farmers to regularly upload information about all their cattle whether healthy or sick at the time. When farmers reported a case of ill health they were provided with a list of differentials and a list of more specific signs and lesions to collect data on to help increase the certainty of the diagnosis. The programme used a Bayes classifier to predict the most likely diagnoses. As for CaDDiS, the information about the project and the web based platform have been removed from the internet.

The above tools have potential for aiding veterinarians, paraprofessionals, and farmers in many contexts. However, to create the most accurate systems large databases of information about clinical signs associated with accurately diagnosed cases is required. Such data sets are very rare. The IDEAL data set is not large but offers an opportunity to investigate whether case definitions can be developed. it has the advantage of having pre and post mortem data on a number of cases of tropical disease. This chapter describes the clinical signs observed in the cohort calves, both how individual clinical signs changed over time, and how clinical signs clustered together within calves. The clinical presentations at death are studied in more detail using multivariate techniques. The focus is on the signs associated with ECF, but the other common causes of death in the cohort (haemonchosis and heartwater) are investigated. Finally, the performance of a published prototype decision support tool is assessed. The tool described in Eisler et al. (2007) was selected as the BoSSS and CaDDiS systems had been removed from the internet and were not available for evaluation.

4.2 Materials and methods

4.2.1 The source of clinical data

All 5-weekly routine visits, and post-mortem visits where the cause of death was of infectious or unknown cause were included in the analyses. Calves that were lost to follow up or died of a non-infectious cause were censored from their last live routine visit. Data from extra clinical episode visits were included in the information associated with post-mortem visits where appropriate and where available.

The clinical data included both qualitative and quantitative measures. Rectal temperature (RT) was taken using a digital thermometer. The thermometer was held against the rectum wall to ensure an accurate measure of core temperature. The packed cell volume (PCV) and total serum protein (TP) were calculated according to methods in chapter 2, section 2.3.1. Automated counts of white blood cells (WBC_sysmex) and lymphocytes (LymNum_sysmex) were made by a Sysmex© haematology analyser. The width of the supra-scapular and pre-crural lymph nodes were measured using calipers.

The qualitative clinical signs were mainly taken from the calf-at-rest data. The page of the form for the collection of calf-at-rest data are in figure 4.2. The animal health assistant was encouraged to examine a body system at a time and record abnormalities within that system. Additional data were extracted from other data tables for the signs icterus, intermandibular or ventral oedema, diarrhoea, pallor, corneal opacity, and haemoglobinurea. These signs were included in the calf at rest table, but were sometimes recorded in alternative tables by the IDEAL data recorder. For example, the presence of diarrhoea was recorded in three places; (1) soiling or diarrhoea in calf-at-rest, (2) soiling on clinical examination, and (3) diarrhoea on faecal examination. If any one of the alternative signs were present, the calf was classified as positive for that sign.

Clinical signs associated with post-mortem visits were either recorded at necropsy, or were reported by the farmer or the local AHA at weekly visits, or by the IDEAL team at a visit prior to death. Blood samples were rarely available from a post-mortem examination and rectal temperature measurements were meaningless, so the quantitative measures were taken from the last visit to the calf if this was within 14 days of the death. However, this was not available in all cases and 25 deaths had no data for rectal temperature, PCV, total serum protein, or white blood cell and lymphocyte counts.

4.2.2 Diagnosis of causes of death

The causes of death were diagnosed as described in chapter 2, sections 2.2.5, and 2.3.5.

7. Inter-visit history & Inspection at Rest: Calf Health

This section will be completed EVERY TIME you visit the calf. For each disorder you tick, enter **1** if you have observed the disorder when inspecting the animal at rest and enter **2** if you have not observed the disorder, but the farmer has observed such disorder during the inter-visit history.

Feeding/Drinking

□ **Normal** □ **Abnormal** □ **Unknown**

If abnormal select from below:

□ Unable to swallow food [ ]
□ Food apprehension [ ]
□ Anorexia [ ]
□ Decreased appetite [ ]
□ Increased water intake [ ]
□ Decreased water intake [ ]
□ Other_____[ ]
□ Not sure / Not done [ ]

Mouth

□ **Normal** □ **Abnormal** □ **Unknown**

If abnormal select from below:

□ Hyperaemia [ ]
□ Haemorrhages [ ]
□ Blisters [Vesicles / Pustules] [ ]
□ Ulcers/erosions [ ]
□ Scabs / Scars [ ]
□ Other_____[ ]
□ Not sure / Not done [ ]

Feet

□ **Normal** □ **Abnormal** □ **Unknown**

If abnormal select from below:

□ Hyperaemia [ ]
□ Haemorrhages [ ]
□ Blisters [Vesicles / Pustules] [ ]
□ Ulcers/erosions [ ]
□ Scabs / Scars [ ]
□ Other_____[ ]
□ Not sure / Not done [ ]

Skin/Coat

□ **Normal** □ **Abnormal** □ **Unknown**

If abnormal select from below:

□ Generalised alopecia [ ]
□ Nodular lesions [ ]
□ Generalised sloughing [ ]
□ Excessive Sweating [ ]
□ Ulcers / Erosions [ ]
□ Scars / Scabs [ ]
□ Other_____[ ]
□ Not sure / Not done [ ]

Eyes

□ **Normal** □ **Abnormal** □ **Unknown**

If abnormal select from below:

□ Ocular Discharge [ ]
□ Corneal Opacity [ ]
□ Blindness [not nervous condition] [ ]
□ Other_____[ ]
□ Not sure / Not done [ ]

Posture

□ **Normal** □ **Abnormal** □ **Unknown**

If abnormal select from below:

□ Arched back [ ]
□ Recumbency [ ]
□ Extended head and neck [ ]
□ Star-gazing [ ]
□ Wide-based stance [ ]
□ Dog-sitting [ ]
□ Lateral positioning of head [ ]
□ Other_____[ ]
□ Not sure / Not done [ ]

Gait

□ **Normal** □ **Abnormal** □ **Unknown**

If abnormal select from below:

□ Lameness [ ]
□ Stiffness [ ]
□ Limping [ ]
□ Swaying hind quarter [ ]
□ Other_____[ ]
□ Not sure / Not done [ ]

Swelling

□ **Normal** □ **Abnormal** □ **Unknown**

If abnormal select from below:

□ Large muscle groups [ ]
□ Joints [ ]
□ Lymph nodes [ ]
□ Ventral thorax [ ]
□ Ventral abdomen [ ]
□ Other_____[ ]
□ Not sure / Not done [ ]

Nervous / Behaviour Change

□ **Normal** □ **Abnormal** □ **Unknown**

If abnormal select from below:

□ Nystagmus [ ]
□ Blindness [nervous condition] [ ]
□ Muscular tremors / muscle twitching [ ]
□ Convulsions [ ]
□ Incoordination / ataxia [ ]
□ High stepping gait [ ]
□ Circling [ ]
□ General weakness [ ]
□ Reduced sensitivity [ ]
□ Paralysis [ ]
□ Hypersensitivity [ ]
□ Restlessness [ ]
□ Lethargy [ ]
□ Aggression [ ]
□ Excessive licking [ ]

Nervous / Behaviour Change

□ Excessive chewing [ ]
□ Excessive salivation/drooling [ ]
□ Excessive bellowing [ ]
□ Other_____[ ]
□ Not sure / Not done [ ]

Respiratory

□ **Normal** □ **Abnormal** □ **Unknown**

If abnormal select from below:

□ Non-foamy nasal discharge [ ]
□ Foamy nasal discharge [ ]
□ Cough [ ]
□ Costo-abdominal respiration [ ]
□ Shallow / rapid breathing [ ]
□ Deep / laboured breathing [ ]
□ Other_____[ ]
□ Not sure / Not done [ ]

Gastrointestinal

□ **Normal** □ **Abnormal** □ **Unknown**

If abnormal select from below:

□ Tenesmus [straining] [ ]
□ Constipation [ ]
□ Hard faeces: Not bloody [ ]
□ Hard faeces: Bloody [ ]
□ Soiling [ ]
□ Diarrhoea: Not bloody [ ]
□ Diarrhoea: Bloody [ ]
□ Regurgitation / Vomiting [ ]
□ Other_____[ ]
□ Not sure / Not done [ ]

Urinary

□ **Normal** □ **Abnormal** □ **Unknown**

If abnormal select from below:

□ Straining [ ]
□ Excessive urination [ ]
□ Water coloured urine [ ]
□ Reddish-tinged urine [ ]
□ Brownish-tinged urine [ ]
□ Increased consistency of urine [>>density] [ ]
□ Other_____[ ]
□ Not sure / Not done [ ]

Ill Thrift

□ **Normal** □ **Abnormal** □ **Unknown**

If abnormal select from below:

□ Weight loss / loss of condition [ ]
□ Cachexy / extreme thinness [ ]
□ Pale mucous membranes [ ]
□ Icterus [ ]
□ Cyanosis [ ]
□ Rough/staring coat. Not bright/shiny [ ]
□ Weakness [not nervous condition] [ ]
□ Other_____[ ]
□ Not sure / Not done [ ]

Figure 4.2: Calf at rest section of calf visit questionnaire. Used for routine, clinical, and post-mortem visits.

4.2.3 Analysis of the clinical sign data

The clinical data consisted of a mix of both descriptions of clinical signs (captured as binary categorical data) and measures of biological parameters (continuous). The aim was to investigate whether there were patterns of clinical expression in the cohort. It was hypothesised that these would correlate well with current knowledge on the diseases known to have affected the calves. However, it was suspected that clinical syndromes, if identified, may have differed from the standard clinical descriptions of disease, as calves were often infected with more than one potential pathogen at any one time.

The aim was to investigate how the clinical parameters, and characteristics of the clinical episodes in particular, changed with age. How prevalent were certain clinical signs in apparently otherwise healthy calves when compared to those experiencing a clinical episode? Also, how did this difference vary by age? Each visit was classified as to whether the calf was suffering from a clinical episode at that visit. It was therefore possible to compare the clinical signs present in clinical episodes and 'normal' visits by age. The population mean of the quantitative measures was calculated for each visit for normal and clinical episode visits. For the qualitative clinical signs, the proportion of visits in which the clinical sign was observed was calculated. These calculations only included the routine visit and post-mortem data. Post-mortem visits were assigned to the week which would have been the next routine visit to the calf. Data from extra clinical visits were not included.

It is rare for a single clinical sign to be pathognomonic for a particular disease. Therefore, clinical signs need to be considered together - the basis of syndromes. Principal components analysis (PCA), multiple correspondence analysis (MCA), and mixed data factor analysis (AFDM) were used to investigate relationships between clinical signs and diagnoses. The methods for these techniques are described in chapter 2, section 2.4.1. The three methods were applied to the clinical data associated with post-mortem visits. The causes of death assigned by the expert panel (see chapter 2, section 2.2.5) were incorporated as supplementary variables, allowing the relationship between signs and disease to be examined. Supplementary variables were not included in dimensions or components, but were able to be associated with these following

analysis to examine relationships between clinical signs and cause of death.

4.2.4 Assessment of prototype clinical decision support tool

The aim was to investigate how the diagnoses of death made by the DST compared with those made by the expert panel as a means of investigating the performance of the tool. To apply the tool to the IDEAL clinical data some re-labelling of clinical signs was required. To provide clinical signs in the format required by the card the IDEAL clinical data were categorised as follows:

- **Anaemia or pallor:** A PCV% of less than 24% (Schlam 2000) at the previous visit if within 14 days or pallor of mucous membranes reported.
- **Anorexia or depression:** Farmer or animal health assistant or visiting IDEAL team reported anorexia, decreased appetite, or lethargy just prior to death.
- **Ataxia or abnormal behaviour:** Farmer or animal health assistant or visiting IDEAL team reported any nervous signs or behaviour change just prior to death.
- **Constipation:** Farmer or animal health assistant or visiting IDEAL team reported constipation, tenesmus or straining, or hard faeces just prior to death.
- **Diarrhoea:** Farmer or animal health assistant or visiting IDEAL team reported soiling or non-bloody diarrhoea just prior to death, or diarrhoea was reported from the inspection of faecal material.
- **Dysentery:** Farmer or animal health assistant or visiting IDEAL team reported bloody diarrhoea just prior to death.
- **Dyspnoea or coughing:** Farmer or animal health assistant or visiting IDEAL team reported coughing, deep laboured breathing, or shallow rapid breathing just prior to death.
- **Haemoglobinuria:** Farmer or animal health assistant or visiting IDEAL team reported reddish tinged or brownish tinged urine just prior to death.

- **Icterus:** Farmer or animal health assistant or visiting IDEAL team reported icterus or yellow discolouration of any tissues just prior to death or at post-mortem examination.
- **Lymph node enlargement:** Farmer or animal health assistant or visiting IDEAL team reported generalised lymph node swelling just prior to death or at post-mortem examination.
- **Pyrexia:** Rectal temperature of more than 40.4^0C in visit within 14 days of death.
- **Staring coat:** Farmer or animal health assistant or visiting IDEAL team reported staring hair coat just prior to death.
- **Stunted growth or pot belly:** None of the clinical signs collected could be categorised as stunted growth or pot belly. This sign was not able to be included in the analysis.
- **Submandibular / ventral oedema:** IDEAL team reported bilateral diffuse swelling or oedema of the inter-mandibular space, or the ventral abdomen either at the visit just prior to death or at post-mortem examination.
- **Weakness:** Farmer or animal health assistant or visiting IDEAL team reported weakness just prior to death.
- **Weight loss:** Farmer or animal health assistant or visiting IDEAL team reported weight loss or loss of condition just prior to death.

No calves were described to have constipation, dysentery, haemoglobinuria, or submandibular or ventral oedema at or prior to their post-mortem visit.

The scores for each disease in each calf were calculated dependent on the clinical signs present. The disease with the highest total score was assigned as the most likely cause, and that with the second highest score the second most likely. If two diseases had the same high score, one was assigned to most, and one to second most likely cause. Prior knowledge of the known causes of death in the cohort pragmatically led to a disease known to have been present being assigned as the most likely cause.

The sensitivity and specificity of the diagnostic support tool (DST) for the diagnosis of East Coast fever was investigated using the cause of death assigned by the IDEAL panel as the gold standard test (primary or contributing cause of death). Only deaths with a known infectious cause were included in the analysis. An inability to reach a diagnosis was sometimes due to a lack of clinical information associated with a death, and it was believed that this would have reduced the performance of the DST.

Sensitivity and specificity were calculated as below:

$$Sensitivity = \frac{No.TruePositives}{No.TruePositives+No.FalseNegatives}$$

$$Specificity = \frac{No.TrueNegatives}{No.TrueNegatives+No.FalsePositives}$$

The DST essentially described syndromes associated with different diseases. MCA was used to investigate whether the clinical signs used in the decision support tool led to similar diagnoses to the expert panel. The diagnoses were included in the MCA as supplementary variables, and the mean value for the calves in each cause of death group was plotted.

The clinical signs from the DST were also incorporated to a logistic regression model for the outcome, ECF death, to see whether this method would improve on that used by the DST card.

4.3 Results

4.3.1 The clinical signs observed in the calves

A total of 62 different clinical signs were observed and included in analyses, in addition to the information collected on five quantitative clinical parameters. A list of all the categorical signs and the number of times that they were observed is in appendix I.1. A total of 3585 visits had no clinical abnormalities observed. Only a small number of visits had more than one clinical sign recorded, and unsurprisingly, these were often those visits classified as clinical episodes (figures 4.3 and 4.4).

Although some clinical signs were common in the cohort, only a rough staring coat

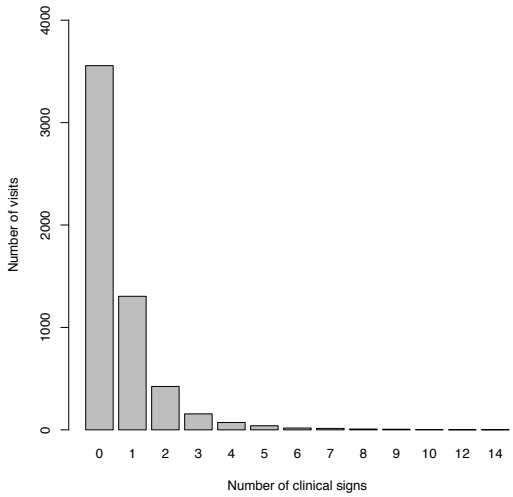

Figure 4.3: Number of clinical signs recorded in each visit

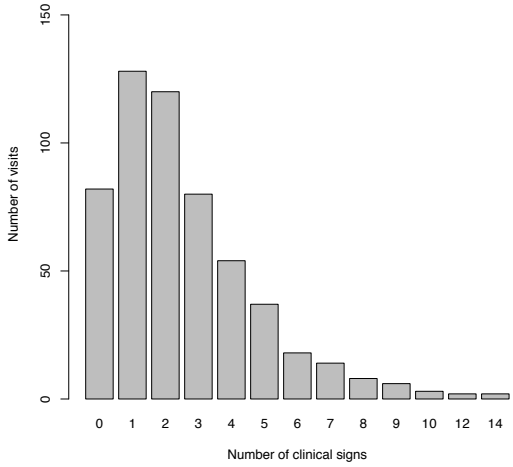

Figure 4.4: Number of clinical signs recorded in each visit only in those visits classified as clinical episodes.

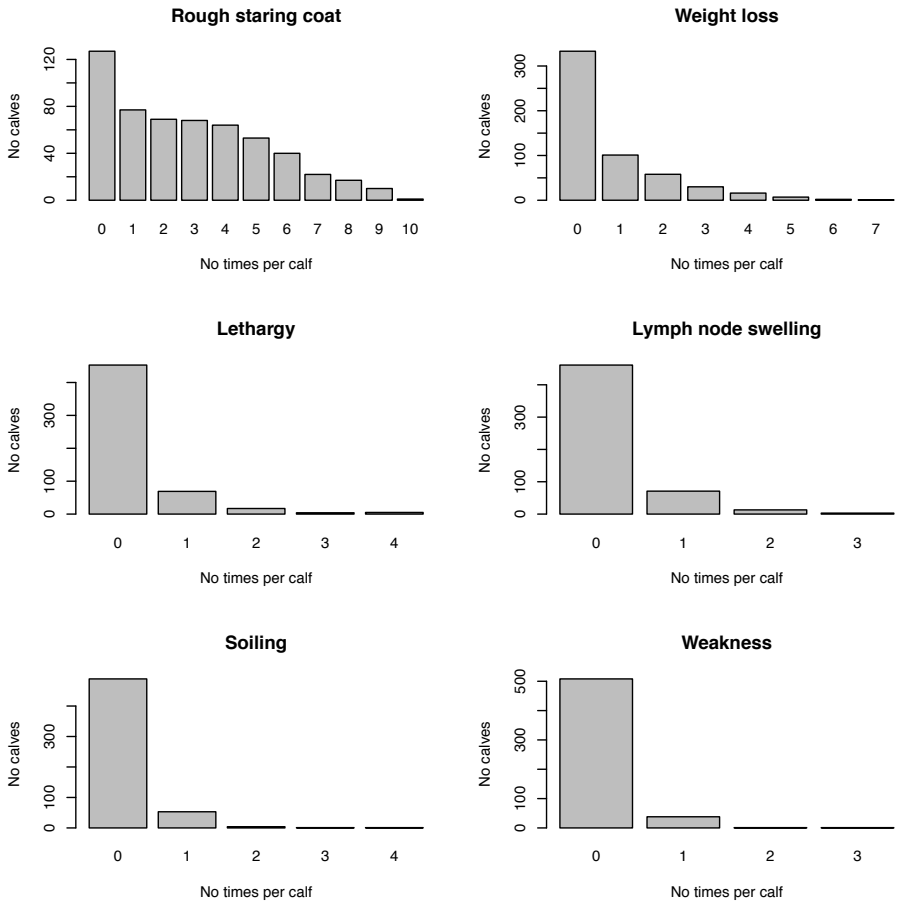

Figure 4.5: Number of times different clinical signs were recorded in a single calf

and weight loss were identified in several visits to the same calf (figure 4.5). The signs that were found repeatedly could be considered as signalling sub-optimal condition in calves, rather than overt clinical disease.

4.3.2 Clinical signs by age

Figures 4.6 to 4.11 compare the mean values for the quantitative clinical parameters by age and between normal and clinical episode routine visits. It should be noted that the 95% confidence intervals on the means for all parameters are wide, and the intervals for the clinical episodes and normal visits overlap. It is possible to discuss trends here, but there is not evidence for statistical differences. It suggests that defining a normal range for these calves may be difficult and possibly innappropriate.

The mean rectal temperature of the calves remained nearly constant at all ages, and sick calves had a consistently higher rectal temperature (figure 4.6). Pyrexia was a common finding associated with clinical disease at all ages. This was inevitable as any visit to a calf with a rectal temperature of 40.5^0C or above was automatically defined as a clinical episode.

The packed cell volumes of calves peaked at week six and then gradually declined towards the final visit at one year old (figure 4.7). The PCV in sick calves was consistently lower than in healthy calves, apart from at the recruitment visit when clinical episodes were rare. There was some divergence at older visits, between healthy and sick calves, suggesting that a lowered PCV was more common in clinical episodes at older ages.

The mean total serum protein in the healthy calves declined from birth to week 21 after which it began to rise slightly (figure 4.8). The total serum protein of the sick calves was consistently lower than for the healthy calves. As for the PCV %, the mean total protein of sick calves diverged from the healthy calves at older visits, suggesting that hypoproteinaemia may have become increasingly important in older clinical episodes.

At a population level, lymph node hyperplasia was a component of clinical episodes only at younger visits (weeks 6 to 21, figures 4.10 and 4.11). Following this there was no difference in mean lymph node width identified in healthy or clinical episode visits.

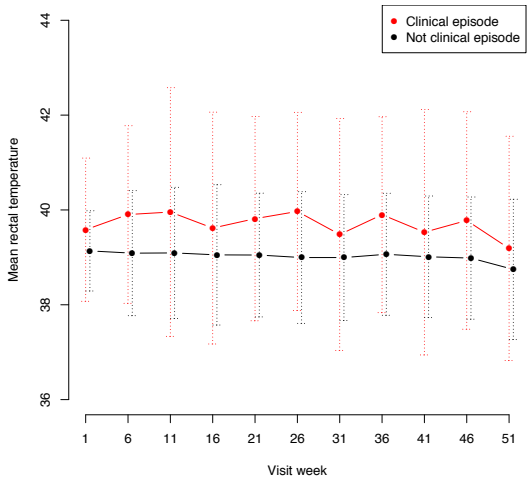

Figure 4.6: Mean rectal temperature in routine visits by whether those visits were classified as clinical episodes or not. Vertical bars are 95% confidence intervals on the estimates.

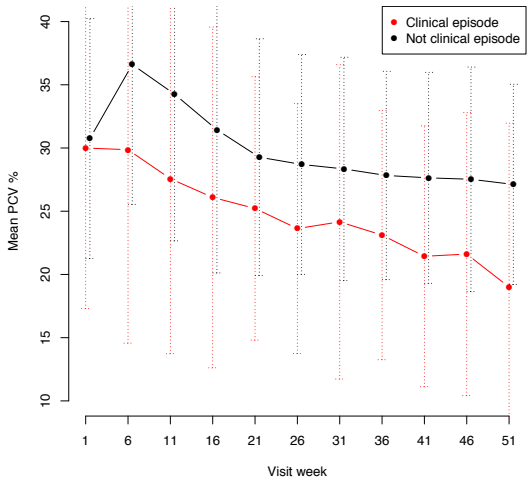

Figure 4.7: Mean PCV % in routine visits by whether those visits were classified as clinical episodes or not. Vertical bars are 95% confidence intervals on the estimates.

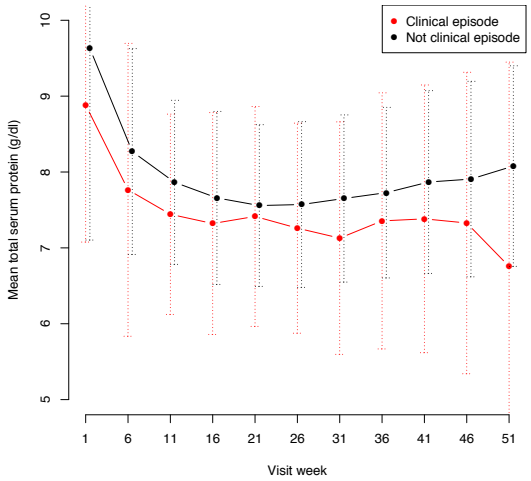

Figure 4.8: Mean total serum protein in routine visits by whether those visits were classified as clinical episodes or not. Vertical bars are 95% confidence intervals on the estimates.

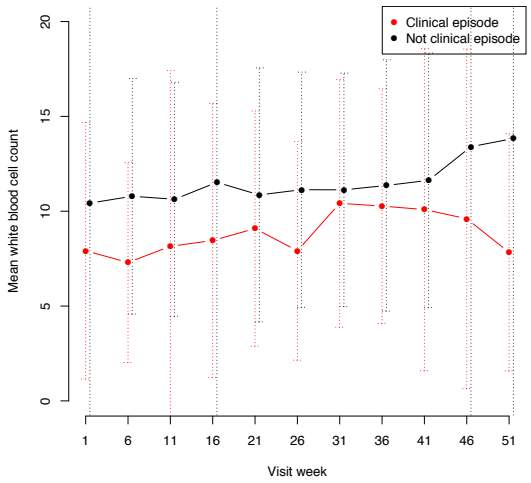

Figure 4.9: Mean white blood cell count in routine visits by whether those visits were classified as clinical episodes or not. Vertical bars are 95% confidence intervals on the estimates.

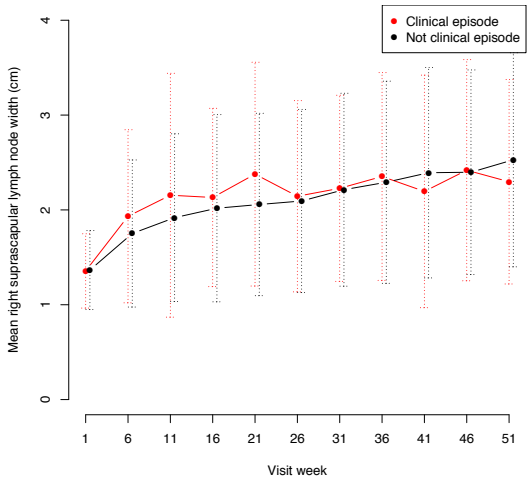

Figure 4.10: Mean right supra-scapular lymph node width in routine visits by whether those visits were classified as clinical episodes or not. Vertical bars are 95% confidence intervals on the estimates.

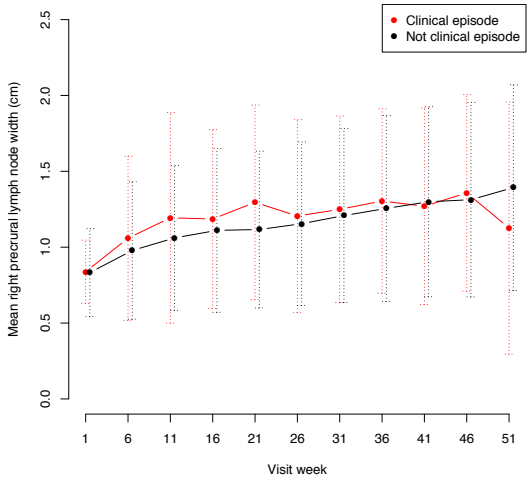

Figure 4.11: Mean right pre-crural lymph node width in routine visits by whether those visits were classified as clinical episodes or not

Rough staring coat was seen most frequently in calves at week 16 after which the number of affected calves gradually declined (figure 4.12). Although more commonly observed in clinical episode visits, the observation of a rough staring coat was also associated with normal visits. From week 16 to the final visit a rough staring coat was observed in between 20 and 40% of healthy visits.

Weight loss or loss of condition was more commonly associated with clinical episode visits, but similar to rough staring coat, it was observed in between 5 and 10% of healthy routine visits from week 16 to the final visit at one year old (figure 4.13).

Lethargy was mainly observed in association with clinical episodes (figure 4.14), and is likely to have contributed to the decision to classify the visit thus. The proportion of clinical visits that involved lethargy declined towards week 21 and then rose again, becoming increasingly common in clinical episodes in older calves.

As was the case for lethargy, generalised lymph node hyperplasia was very rarely associated with healthy visits (figure 4.15), and again was likely to have made a significant contribution to a visit being classified as a clinical episode. Lymph node hyperplasia was most common in the clinical episodes in the younger calves, and declined in importance at the older visits, supporting the findings of figures 4.10 and 4.11.

The opposite was the case for a decreased appetite and soiling, which became increasingly common in older clinical episodes (figures 4.16 and 4.17).

4.3.3 The relationship between clinical signs and diagnosis

MCA, PCA, and AFDM were used to investigate the relationships between clinical signs and cause of death. The causes of death were added to all models as supplementary variables.

Of the 548 calves recruited to the study, 57 died from a known infectious cause, 11 from an unknown infectious cause, and six were not able to be identified as infectious or non-infectious. The clinical data from all these deaths were included in analyses.

Of the calves with known infectious causes, 32 died from East Coast fever as the

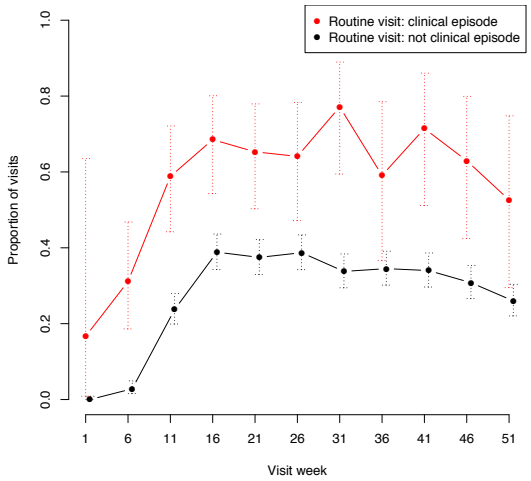

Figure 4.12: Proportion of calves at visits classified as having a rough staring coat and whether those visits were classified as clinical episodes or not. Vertical bars are 95% confidence intervals on the estimates.

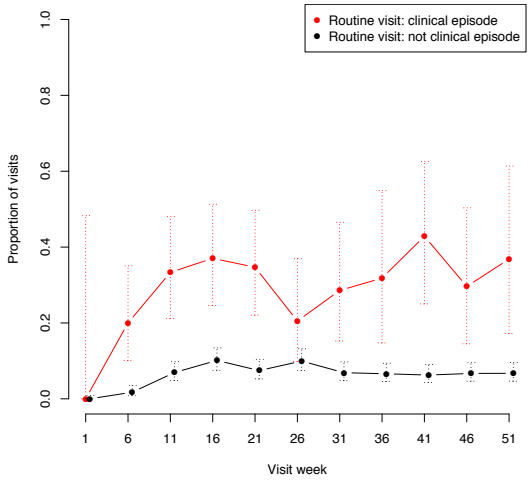

Figure 4.13: Proportion of calves at visits classified as experiencing weight loss or loss of condition and whether those visits were classified as clinical episodes or not. Vertical bars are 95% confidence intervals on the estimates.

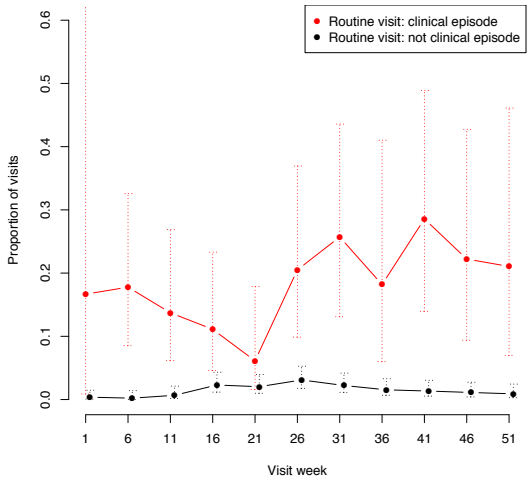

Figure 4.14: Proportion of calves at visits classified as experiencing lethargy and whether those visits were classified as clinical episodes or not. Vertical bars are 95% confidence intervals on the estimates.

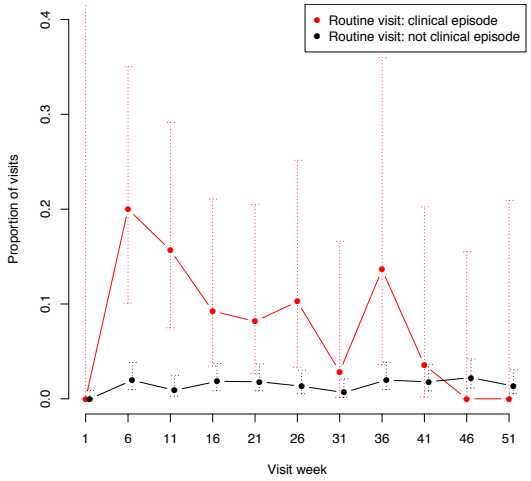

Figure 4.15: Proportion of calves at visits classified as experiencing generalised peripheral lymph node swelling and whether those visits were classified as clinical episodes or not. Vertical bars are 95% confidence intervals on the estimates.

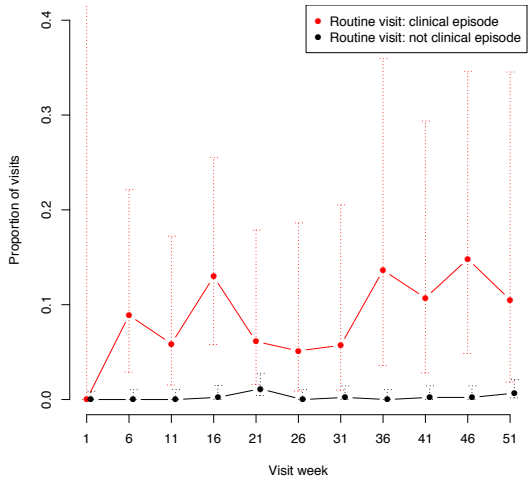

Figure 4.16: Proportion of calves at visits classified as having a decreased appetite and whether those visits were classified as clinical episodes or not. Vertical bars are 95% confidence intervals on the estimates.

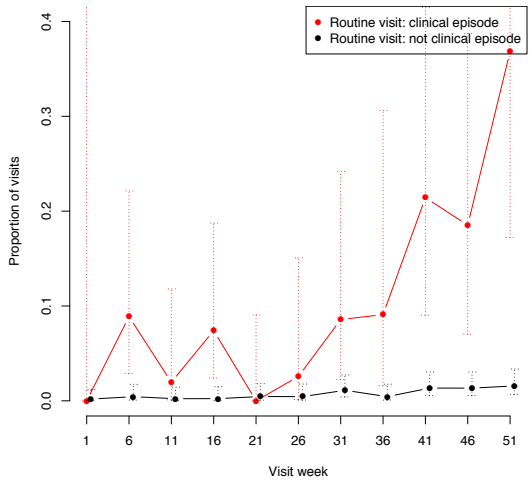

Figure 4.17: Proportion of calves at visits classified as having soiled hind quarters from loose faeces or diarrhoea and whether those visits were classified as clinical episodes or not. Vertical bars are 95% confidence intervals on the estimates.

primary cause of death along with one case of turning sickness (the cerebral form of *Theileria parva* infection) , nine of haemonchosis, six of heartwater, two of a systemic pyogenic bacterial infection (*Arctinomyces pyogenes* and *Arcanobacterium* spp.), and one each of babesiosis, bacterial pneumonia, viral pneumonia, black quarter (clostridiosis), rabies virus, and salmonellosis.

Of the East coast fever deaths, trypanosomiasis was believed to have contributed to two deaths, helminthiasis to three, haemonchosis to five, rotavirus to one, and malnutrition to one death. Of the haemonchosis deaths, helminthiasis was believed to have contributed to four deaths, lung worm to one, and theileriosis to two deaths. East coast fever contributed to one heartwater death, and concurrent lead poisoning was found in another.

PCA was used to derive principal components that represented the covariance of the quantitative clinical parameters within individuals. These were then associated with causes of mortality. The eigenvalues and the % variance represented by the principal components are shown in table 4.1. The correlation between the parameters in the 1st, 2nd, and 3rd principal components and the relationships and distribution of individuals derived from the first three principal components are shown in figures 4.18, and 4.19.

Analysis of the first three components allows a broad description of the relationship between the quantitative signs and the three common causes of death.

Component one differentiated heartwater cases from ECF and haemonchosis cases, and was associated with a relatively higher PCV%, and TP, and white blood cell and lymphocyte count when compared to ECF and haemonchosis cases.

Lower rectal temperature and total serum protein, and higher white blood cell and lymphocyte counts significantly contributed to component 2. This component was significantly associated with haemonchosis deaths, which had relatively lower rectal temperature and PCV, and higher white blood cell and lymphocyte count. This was in contradiction to component one that associated haemonchosis with lower white blood cell and lymphocyte counts. However, the plots of components one and two, and two and three suggest that haemonchosis cases had a higher white blood cell and lymphocyte count when compared to ECF and heartwater cases.

Rectal temperature did not significantly contribute to the first component, but plots show that ECF cases had a relatively higher rectal temperature when compared with haemonchosis cases.

Component three was significantly associated with an increase in rectal temperature and white blood cell count, and a decrease in PCV%. It was not strongly associated with any of the particular causes of death.

The first three principal components explained 82.6% of the variation between individuals, suggesting that the quantitative variables did co-vary. However, when individuals were plotted according to the first three principal components they were not separated by cause of death.

Table 4.1: Contributions of the principal components towards explaining the variation between individuals. Quantitative data associated with post-mortem visits.

	eigenvalue	percentage of variance	cumulative percentage of variance
comp 1	2.12	42.45	42.45
comp 2	1.25	24.98	67.43
comp 3	0.76	15.14	82.58
comp 4	0.61	12.27	94.85
comp 5	0.26	5.15	100.00

MCA was used to derive dimensions that best described the variation between individuals according the the clinical signs associated with their deaths. The eigenvalues and the percentage of variance explained by each component is in table 4.2. The first four dimensions were investigated. These explained 32.8% of the total variance.

The clinical signs significantly contributing to dimension one in the positive direction were respiratory problems, lethargy, ocular discharge, anorexia, weakness, interstitial lymphoblastosis, and lymph node hyperplasia. The absence of signs formed the negative component of dimension one.

The clinical signs significantly contributing to dimension two in the positive direction were weight loss, a rough staring coat, weakness, and gelatinisation of the renal or coronary groove fat, and in the negative direction were lymph node hyperplasia, froth in the bronchial tree, respiratory problems, and interstitial lymphoblastosis.

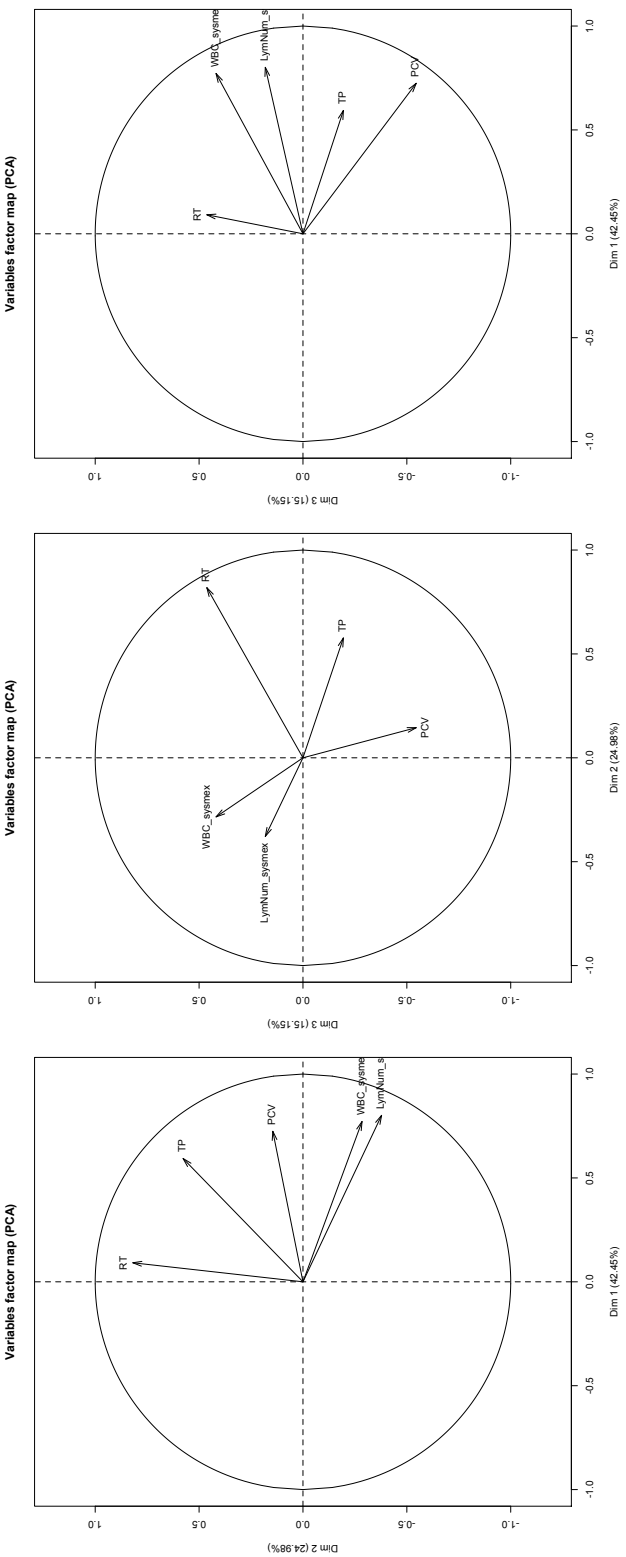

Figure 4.18: Results from PCA. Correlation circles for dimensions 1, 2, and 3. RT = rectal temperature, WBC_sysmex = white blood cell count taken from sysmex haematology reader, LymNum_sysmex = lymphocyte count from sysmex haematology reader, TP = total serum protein, PCV = packed cell volume.

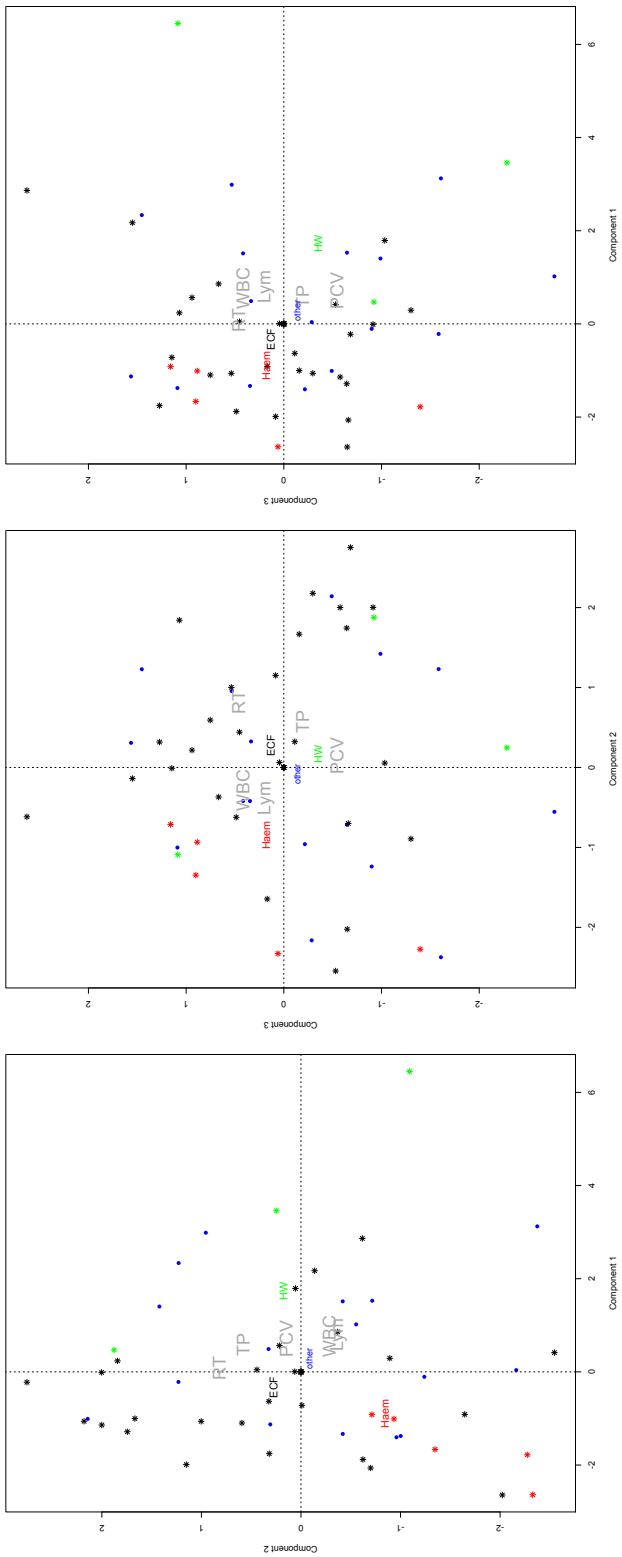

Figure 4.19: Results from PCA. Individuals plotted by dimensions 1, 2, and 3. Coloured according to cause. Black asterisk = ECF cases, red asterisk = haemonchosis cases, green asterisk = heartwater cases, blue asterisk = deaths due to other causes. WBC = white blood cell count, RT = rectal temperature, Lym = lymphocyte count, TP = total serum protein, PCV = packed cell volume.

The clinical signs significantly contributing to dimension three in the positive direction were nervous signs, icterus, weakness, excess peritoneal fluid, and corneal opacity, and in the negative direction were froth in the bronchial tree, gelatinisation of the renal or coronary groove fat, interstitial lymphoblastosis, pethechial haemorrhage of the mucous membranes, weight loss, and a rough staring coat.

Several signs were clustered in the middle of the plots and can not be differentiated. They did not significantly contribute to the MCA dimensions and so were not useful for differentiating clinical differences between calves. They could be considered as more general signs associated with mortality, or non-specific signs.

Figures 4.20, 4.21, or 4.22 show the distribution of individuals and their cause of death, and the relationship between the individuals and clinical signs. The calves with the same cause of death were somewhat, but not entirely clustered by the variation in their clinical signs. The signs associated with the three main causes of death were as expected.

Table 4.2: Contributions of each of the dimensions towards explaining the variation between individuals (MCA). Qualitative data associated with post-mortem visits.

	eigenvalue	percentage of variance	cumulative percentage of variance
dim 1	0.14	13.07	13.07
dim 2	0.11	10.81	23.87
dim 3	0.09	8.95	32.82

The quantitative and qualitative clinical signs were integrated into a single mixed data factor analysis. The causes of death were included as supplementary variables and were further subdivided to include contributing causes of death. The aim was to build on the information from the MCA and PCA analysis to work towards a syndromic description of ECF death. Mixed data factor analysis was used to derive dimensions that best described the variance in the clinical data (both quantitative and qualitative). The first three dimensions were examined, and their associated eigenvalues and the percentage of variance explained by each are in table 4.3.

Dimension one was associated with an increase in all quantitative clinical parameters (figure 4.23). The signs that most contributed to the first dimension were the white blood cell count, respiratory problems, PCV%, and total serum protein.

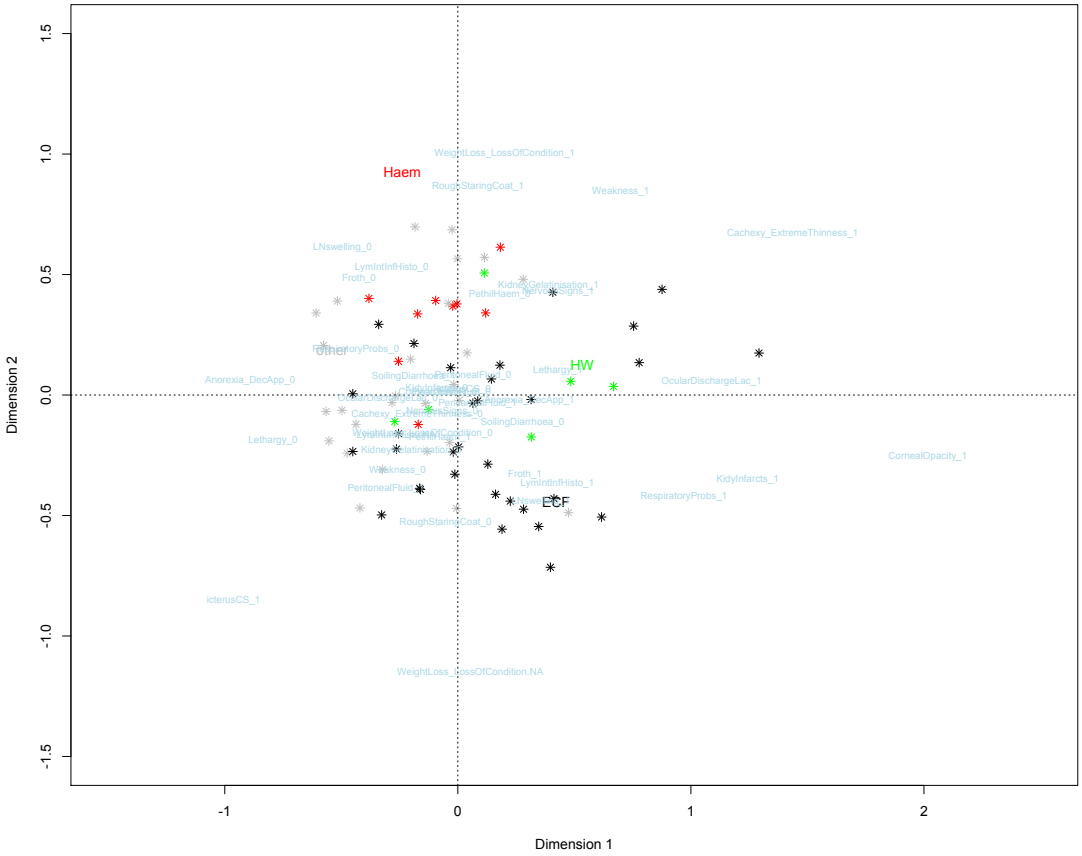

Figure 4.20: MCA for clinical signs. Dimension 1 and 2 with causes of death as supplementary variables. Black asterisk = ECF cases, red asterisk = haemonchosis cases, green asterisk = heartwater cases. Clinical signs in light blue.

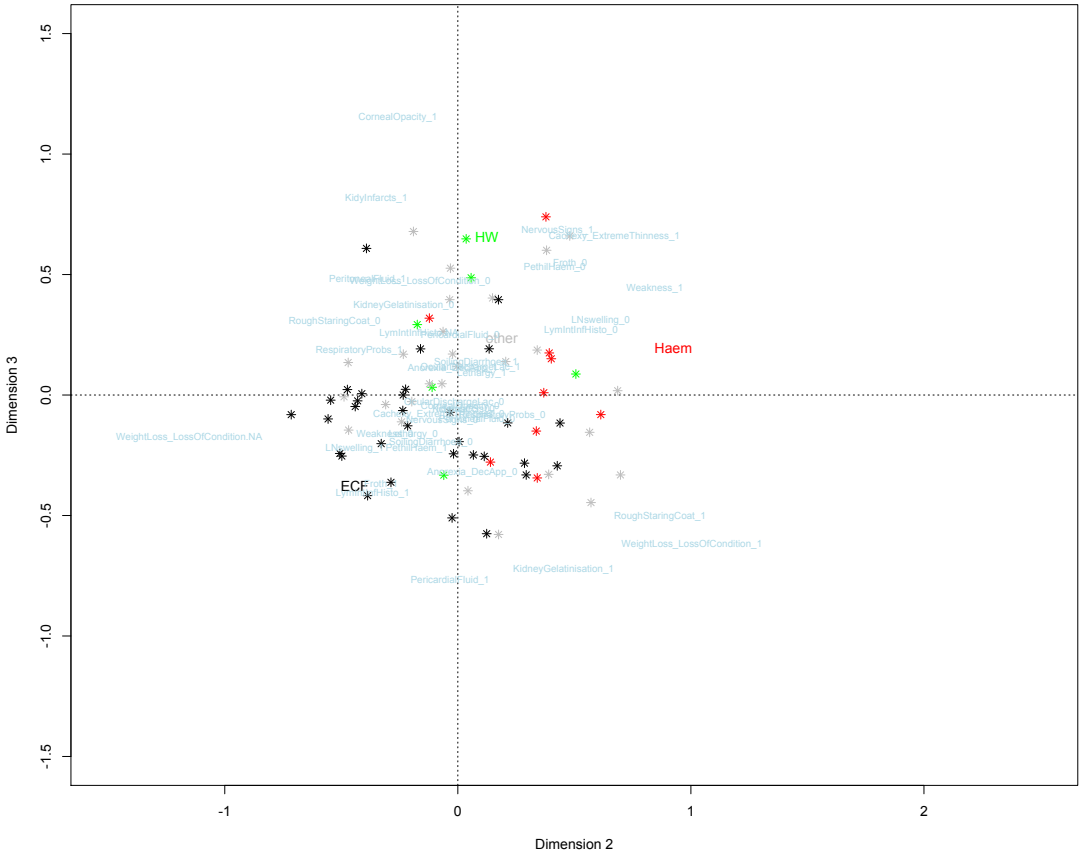

Figure 4.21: MCA for clinical signs. Dimension 2 and 3 with causes of death as supplementary variables. Black asterisk = ECF cases, red asterisk = haemonchosis cases, green asterisk = heartwater cases. Clinical signs in light blue.

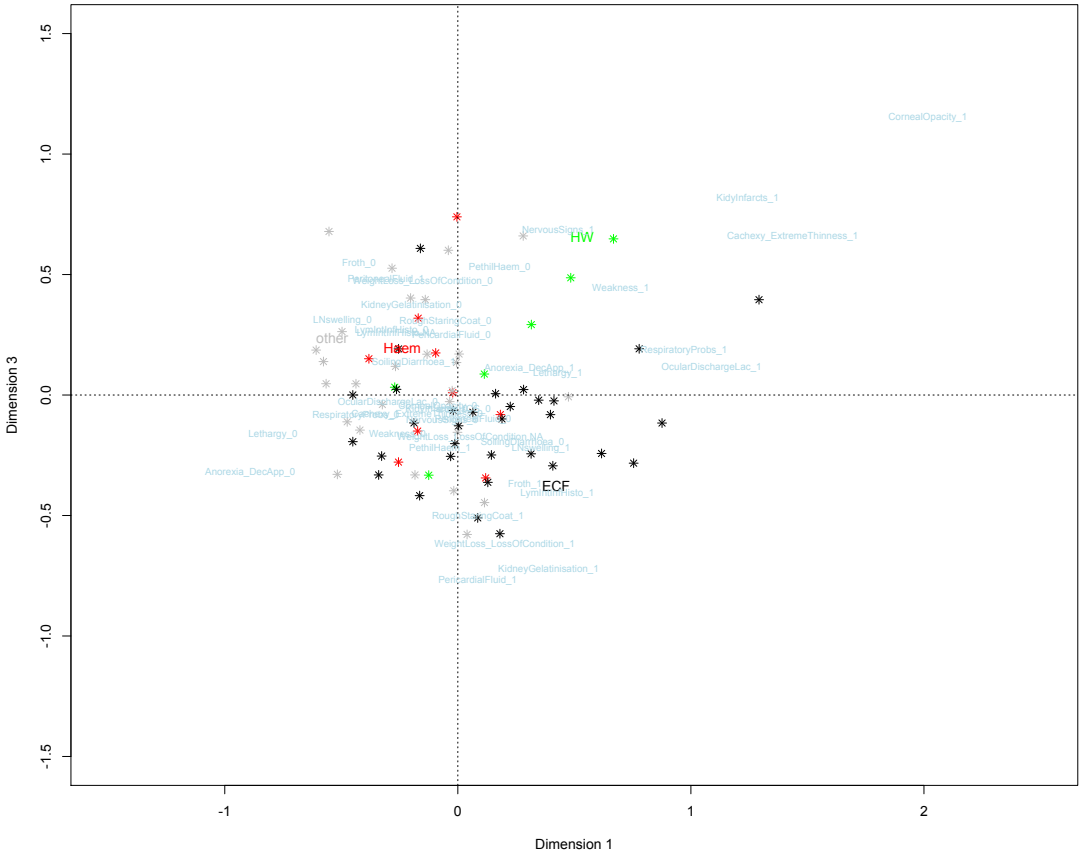

Figure 4.22: MCA for clinical signs. Dimension 1 and 3 with causes of death as supplementary variables. Black asterisk = ECF cases, red asterisk = haemonchosis cases, green asterisk = heartwater cases. Clinical signs in light blue.

The signs that most contributed to the second dimension were weight loss, gelatinisation of the peri-renal or coronary groove fat, rough staring coat, weakness, and lethargy. Movement from negative to positive along dimension two was associated with a decrease in PCV and total serum protein.

The signs that most contributed to the third dimension were lymph node hyperplasia, lymphocyte and white blood cell counts, nervous signs, froth in the bronchial tree, and rectal temperature. Movement along dimension three from negative to positive was associated with a decreasing rectal temperature and an increasing white blood cell and lymphocyte count.

Although the amount of variation explained by the first three dimensions was similar, the individual calves are more distinctly separated into the three common causes of death than when just the quantitative measures or just the qualitative signs were analysed.

It can be seen from figures 4.24 and 4.26 that haemonchosis cases were associated with weakness, staring coat, extreme thinness, and weight loss and loss of condition a relatively higher white blood cell and lymphocyte count, and a relatively lower rectal temperature, total serum protein, and PCV.

ECF was associated with lymph node hyperplasia, respiratory problems, froth in the bronchial tree, and a relatively higher rectal temperature and a relatively lower lymphocyte and white blood cell count and PCV. Heartwater was associated nervous signs, and with a relative increase in the quantitative parameters.

Table 4.3: Contributions of each of the AFDM components towards explaining the variation between individuals. Qualitative and quantitiative data associated with post-mortem visits.

	eigenvalue	percentage of variance	cumulative percentage of variance
comp 1	3.37	12.49	12.49
comp 2	2.74	10.13	22.62
comp 3	2.57	9.53	32.15

In summary, the multivariate techniques identified causes of death to be associated with the expected clinical signs, but significant overlap between causes and variation in clinical presentation within causes was found.

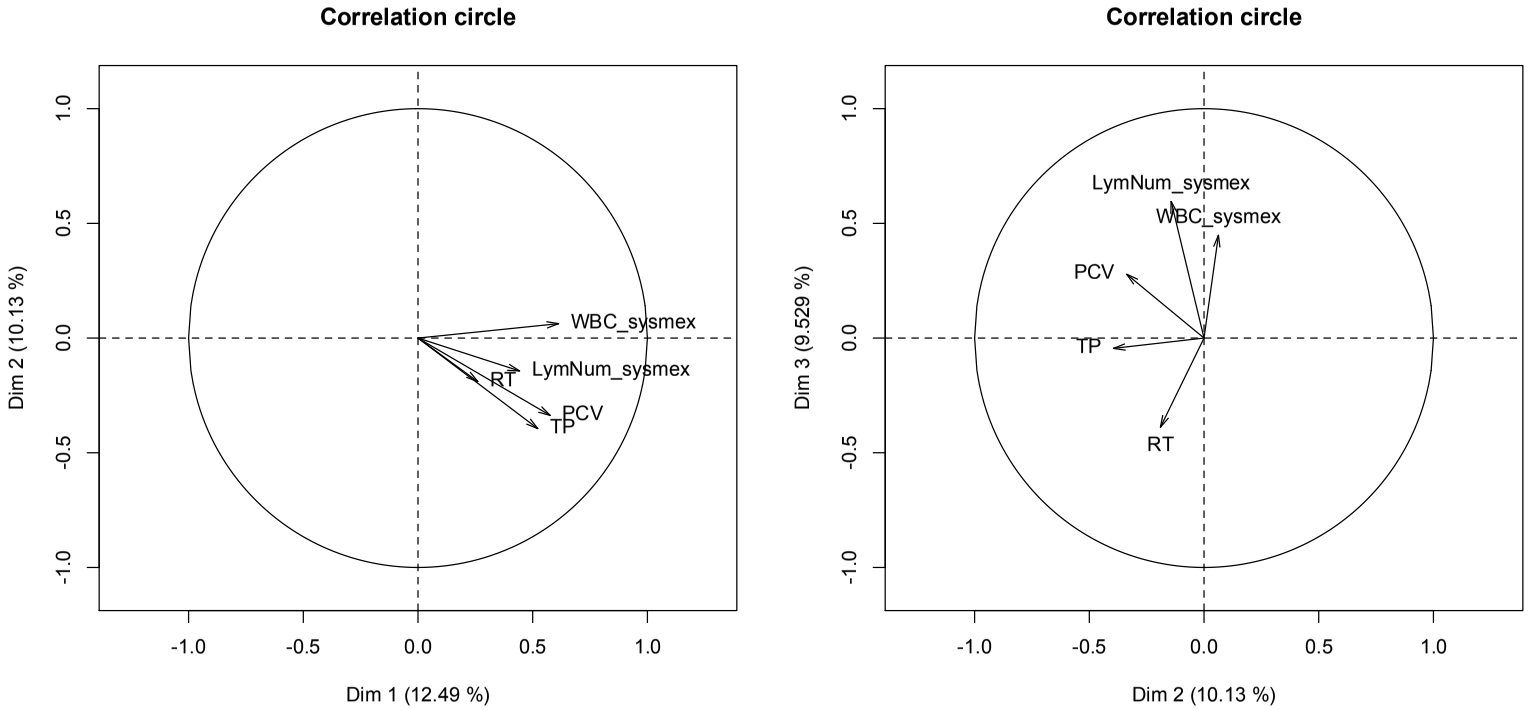

Figure 4.23: Correlation plot for the quantitative variables included in mixed data factor analysis of clinical signs associated with post-mortems. RT = rectal temperature, WBC_sysmex = white blood cell count taken from sysmex haematology reader, LymNum_sysmex = lymphocyte count from sysmex haematology reader, TP = total serum protein, PCV = packed cell volume.

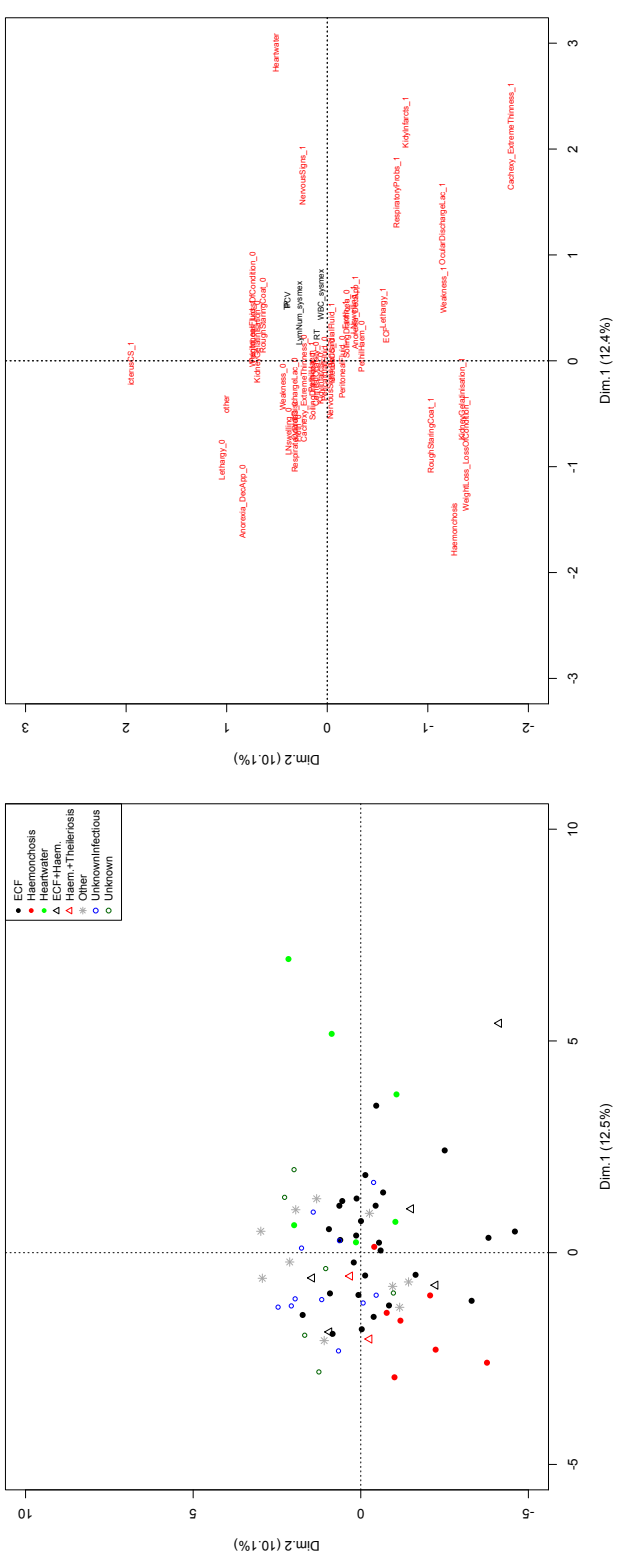

Figure 4.24: Calves and their causes of death plotted by their inter-relationship according to clinical signs (AFDM). Dimensions 1 and 2. RT = rectal temperature, WBC_sysmex = white blood cell count taken from sysmex haematology reader, LymNum_sysmex = lymphocyte count from sysmex haematology reader, TP = total serum protein, PCV = packed cell volume.

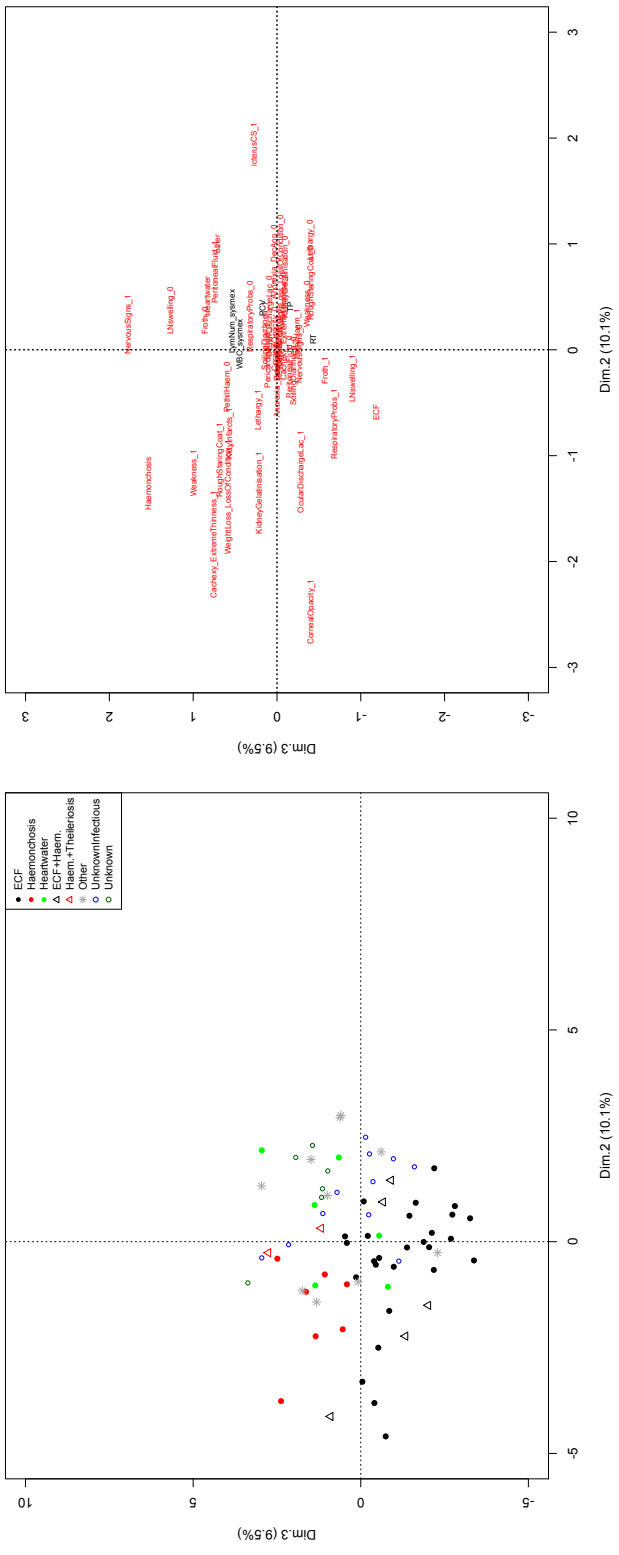

Figure 4.25: Calves and their causes of death plotted by their inter-relationship according to clinical signs (AFDM). Dimensions 2 and 3. RT = rectal temperature, WBC_sysmex = white blood cell count taken from sysmex haematology reader, LymNum_sysmex = lymphocyte count from sysmex haematology reader, TP = total serum protein, PCV = packed cell volume.

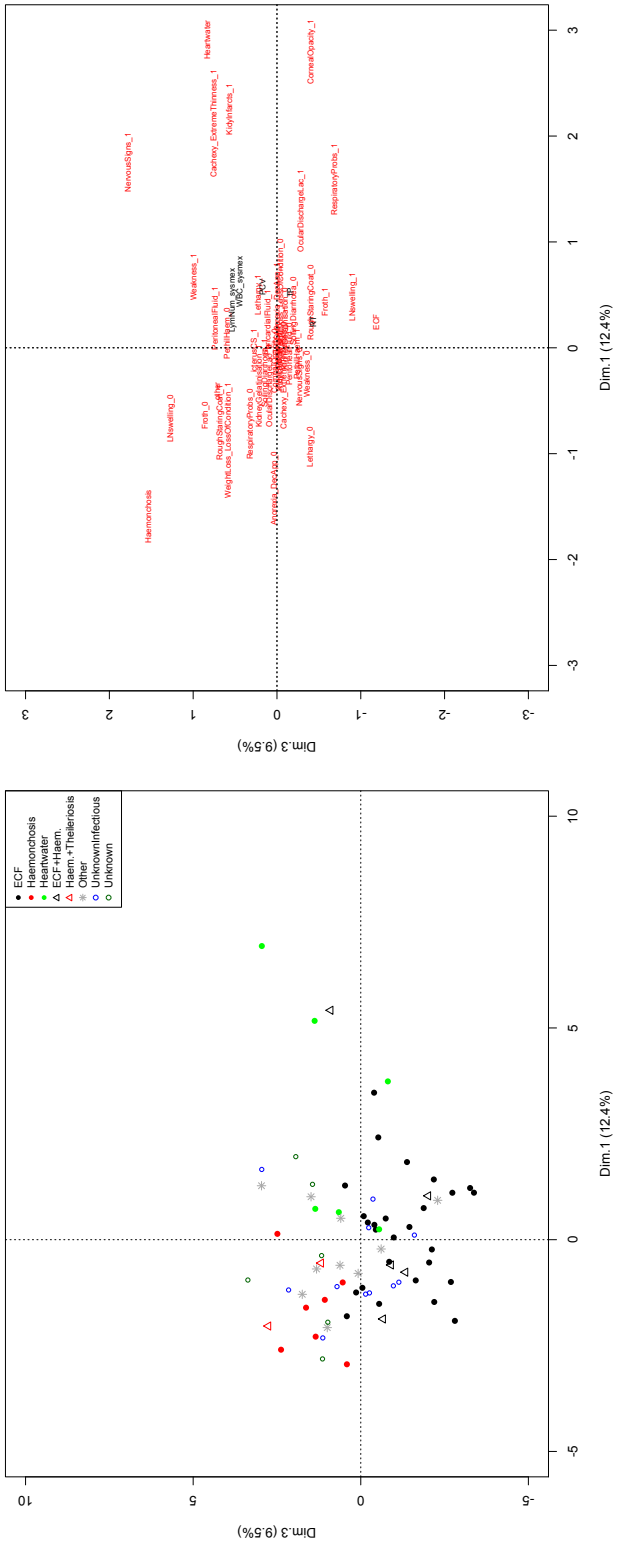

Figure 4.26: Calves and their causes of death plotted by their inter-relationship according to clinical signs (AFDM). Dimensions 1 and 3. RT = rectal temperature, WBC_sysmex = white blood cell count taken from sysmex haematology reader, LymNum_sysmex = lymphocyte count from sysmex haematology reader, TP = total serum protein, PCV = packed cell volume.

4.3.4 Performance of prototype decision support tool

The decision support tool was used to find the most likely diagnosis in 74 cases of calf death. It diagnosed 30 cases of theileriosis (ECF), 24 cases of trypanosomiasis, and 10 cases of cowdriosis (heartwater). In one calf, three diseases had a joint equal score (cowdriosis, fasciolosis, and schistosomiasis), and in one case, none of the signs were present and no specific disease was identified. The data used in and the diagnoses made by the DST for each calf are in appendix I.3.

The diagnosis of ECF by the DST was assessed, but only including deaths with a known cause. The diagnosis of ECF by the DST and the expert panel are compared in table 4.4. The specificity of the DST for the identification of ECF cases was high at 83%, but the sensitivity was only 59%.

Table 4.4: 2x2 table comparing the diagnosis made by the decision support tool with the diagnosis made by the expert panel

	Expert ECF -ve	Expert ECF +ve
DST ECF -ve	19	14
DST ECF +ve	4	20

There were 14 ECF cases that were mis-diagnosed by the DST. In 11 calves the most likely cause identified by the DST was trypanosomiasis. The misdiagnoses in this group were because the calves were frequently anaemic, a clinical sign with a high score for trypanosomiasis. The calves were also not observed as pyrexic, a high scoring clinical sign for ECF. The last live visit varied in its time from death, and it is possible that calves were not observed during the period of pyrexia. It is also possible that the cut-off for anaemia was set too high leading to false positives. However, 10 of the 11 calves classified as being anaemic had a very low PCV of 20% or less. Therefore, it appears that the DST was over-diagnosing trypanosomiasis in this population. However, it is possible that the IDEAL panel was under diagnosing this disease.

The DST was applied to 17 calf deaths that were unable to be definitively diagnosed by the expert panel. The DST identified these as six cases of ECF, three cases of heartwater, five cases of parasitic gastroenteritis (PGE), and two cases of trypanosomiasis. The case that was not diagnosed by the decision support tool was also not diagnosed by the expert committee. In the cases where the expert panel was unable

to come to a decision it is not possible to know whether the DST made correct diagnoses.

MCA was applied to the clinical signs included in the DST to derive dimensions that best described the variation in clinical presentation of the calves. It was then possible to examine the relationship between the clinical signs and the diagnoses made by the DST and the diagnoses made by the expert panel.

Figures 4.27 and 4.28 show the relationship between the individual calves and their causes of death with the dimensions derived from the DST clinical signs. Figure 4.29 shows the individual diagnoses for each calf and their inter-relationships according to the clinical signs used by the DST.

The DST does not appear to be able to differentiate between trypanosomiasis and haemonchosis cases, which is not surprising as anaemia is a common component of both these diseases, and the DST was not designed to diagnose haemonchosis. The mean values for the cases grouped by the diagnosis of theileriosis / ECF (DST / expert committee) are close together. The mean values of the heartwater / cowdriosis cases (expert committee / DST) are not close, suggesting that the DST is misdiagnosing these cases, and possibly not applying appropriate signs to heartwater diagnosis. However, dimension three does cluster the heartwater cases diagnosed by the expert panel and the DST by the presence of nervous signs.

When the individuals are plotted according to dimensions one, two, and three (figure 4.29), they are not grouped by cause. This suggests that the clinical signs used in the DST did not describe clinical variation between causes of death. However, the DST explicitly states that clinical signs are shared between causes. This may explain the overlap between individuals with different diagnoses.

Logistic regression modelling was used to investigate the association between the clinical signs used in the decision support tool and ECF death in the cohort. The summary of the full model including all clinical signs is in table 4.5, and the summary of the final model is in table 4.6. It can be seen that lymph node hyperplasia and anaemia were significantly associated with ECF death. Pyrexia was marginally significant. The final model was used to predict outcome in the same set of calves. There was not enough data available to split the data to allow some to be used for

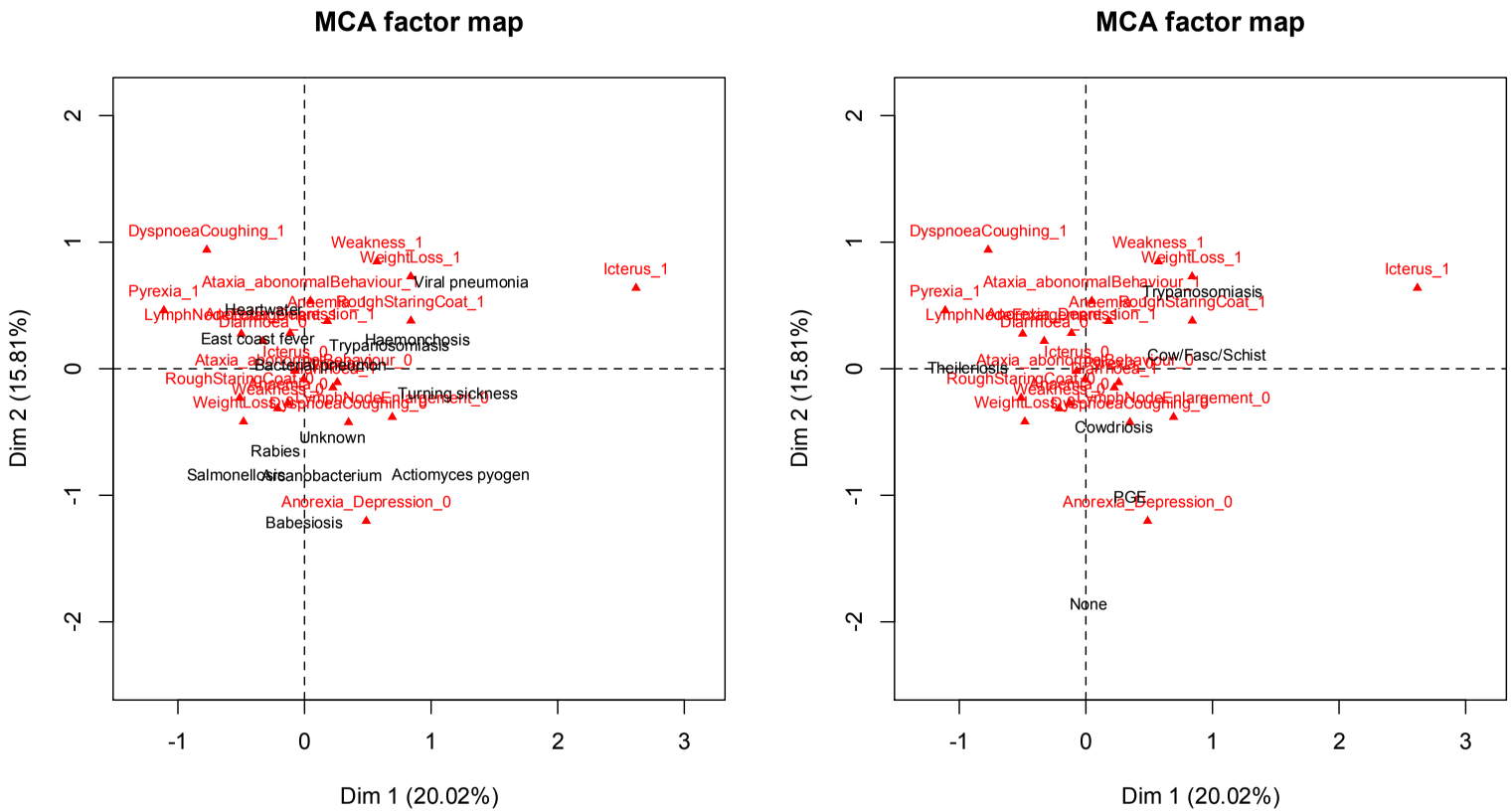

Figure 4.27: Calves and their causes of death plotted by their inter-relationship according to clinical signs (AFDM). Dimensions 1 and 2. The left hand plot shows the diagnoses made by the IDEAL panel, and the right hand plot, those made by the DST. The term ECF was used by the IDEAL expert panel and was considered to be equivalent to the diagnosis of theileriosis made by the DST. The term heartwater was used by the expert panel and was considered to be equivalent to the diagnosis of cowdriosis made by the DST

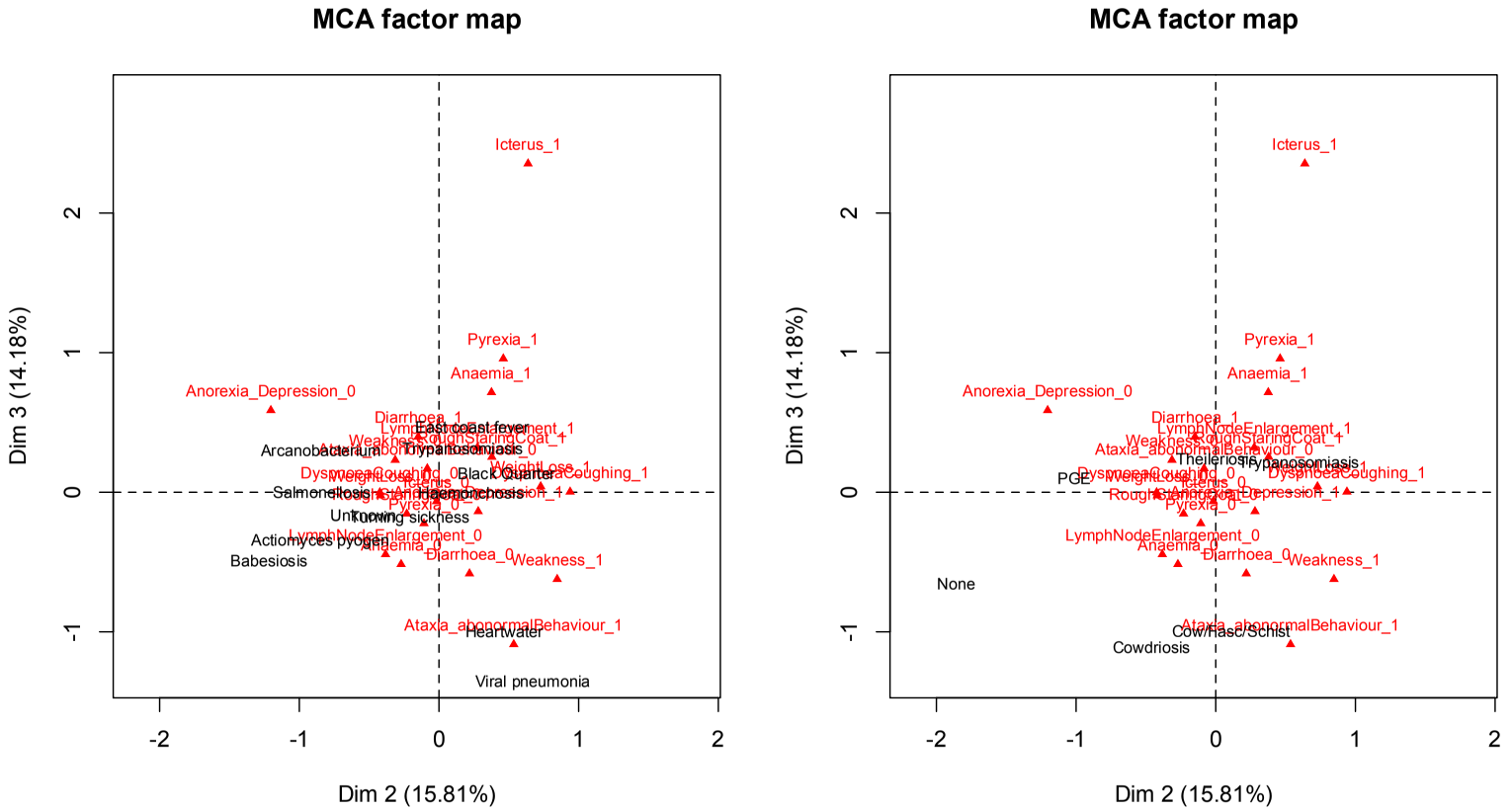

Figure 4.28: Calves and their causes of death plotted by their inter-relationship according to clinical signs (AFDM). Dimensions 2 and 3. The left hand plot shows the diagnoses made by the IDEAL panel, and the right hand plot, those made by the DST. The term ECF was used by the IDEAL expert panel and was considered to be equivalent to the diagnosis of theileriosis made by the DST. The term heartwater was used by the expert panel and was considered to be equivalent to the diagnosis of cowdriosis made by the DST

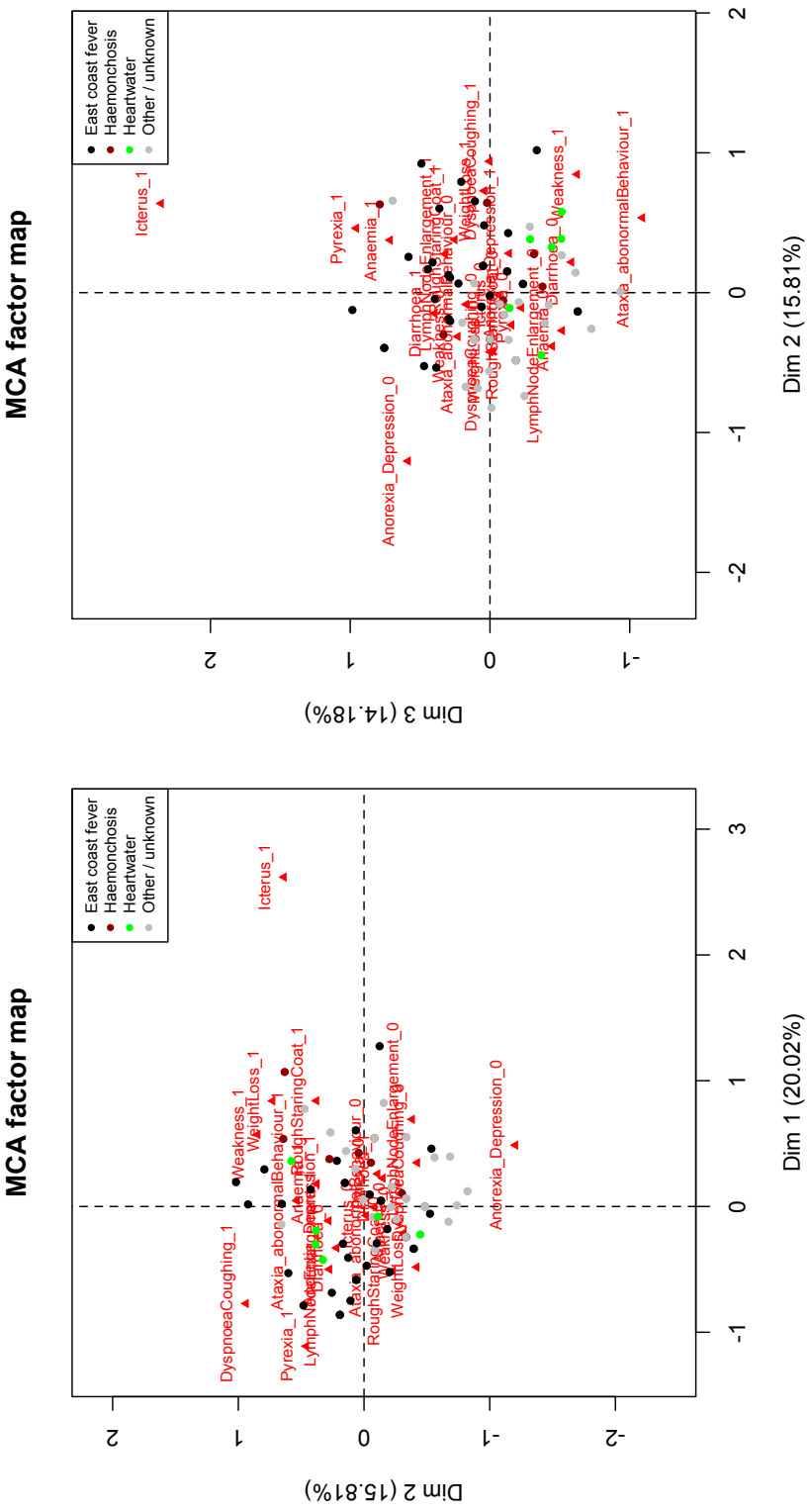

Figure 4.29: Calves and their causes of death (expert panel) plotted by their inter-relationship according to clinical signs incorporated to the DST.

model development and some to be used for model testing. The cut-off for the predicted odds used to define a calf as an ECF case was 0.5. The diagnoses defined by the model and the actual diagnoses are compared in table 4.7. The sensitivity of the logistic regression model for the prediction of ECF was 64% with a specificity of 90%.

This suggests that these three signs included in a logistic regression model acted as a better decision support tool when compared to the card based tool. However, this method does not allow the likelihood of other diseases to be considered. This is a significant disadvantage compared to the card based DST.

Table 4.5: Maximum model predicting cases of ECF using clinical signs taken from the DST

	Estimate	OR	OR_CI2.5	OR_CI97.5	Pvalue
(Intercept)	-1.24	0.29	0.04	1.68	0.191
Anaemia	1.59	4.92	1.38	20.34	0.019
Anorexia or depression	-0.42	0.66	0.11	3.85	0.637
Ataxia or abonormal behaviour	-0.71	0.49	0.06	2.93	0.459
Diarrhoea	-0.51	0.60	0.16	2.13	0.431
Dyspnoea or coughing	0.29	1.34	0.29	6.03	0.705
Icterus	0.36	1.44	0.04	54.62	0.828
Pyrexia	1.54	4.69	0.74	47.23	0.131
Rough staring coat	-0.71	0.49	0.10	2.36	0.378
Lymph node enlargement	1.86	6.44	1.78	27.25	0.007
Weight loss	-0.12	0.88	0.15	4.75	0.886
Weakness	-0.20	0.82	0.16	3.92	0.801

Table 4.6: Parsimonious model predicting cases of ECF using clinical signs taken from the DST

	Estimate	OR	OR_CI2.5	OR_CI97.5	Pvalue
(Intercept)	-2.26	0.10	0.03	0.30	$<$0.001
DST_Anaemia	1.29	3.62	1.15	12.40	0.031
DST_Pyrexia	1.71	5.53	1.09	43.95	0.06
DST_LymphNodeEnlargement	2.06	7.83	2.43	29.52	0.001

Table 4.7: 2x2 table comparing the true diagnosis and the finding of the logistic regression model

	Model ECF -ve	Model ECF +ve
ECF -ve	36	4
ECF +ve	12	22

4.3.5 Diagnoses by the attending clinician, the pathologist, and the expert panel

The expert panel were provided with all the available information for each of 82 calves in receipt of a post-mortem and a diagnosis was sought in each case. Six of the 82 died from an unknown cause. Eight died from non-infectious causes (trauma, mismothering, starvation, or poisoning).The remaining 68 died from infectious causes. For 11 of the 68 it was not possible to identify a specific cause.

Of the 19 calves with no histological examination data available, the expert panel were able to attribute a specific cause to 11 (five cases of ECF, two haemonchosis, one heartwater, one trypanosomiasis, and one case of generalised abcessation (*Arcanobacterium*). Four calves that were not able to be diagnosed by expert panel were missing a contemporary post-mortem report from the attending vet.

As a means of assessing how well the attending clinician was able to diagnose the cause of death without histological examination, the tentative diagnosis made by the attending vet at the time of the post-mortem was compared to the diagnosis made by the pathologist following histological examination of tissues. The pathologist had the PM report available at the time of histological diagnosis.

A total of 27 calves were diagnosed with East Coast Fever by the expert panel and had a histological diagnosis of interstitial lymphoblastic infiltration, a sign pathognomonic for ECF. Of these, seven had no contemporary post-mortem report or diagnosis and it would have been unlikely that the expert panel would have reached a definitive diagnosis without results from histological examination. 20 of the calves with interstitial lymphoblastic infiltration had a contemporary report associated with their post-mortem. Of these, 14 cases had been diagnosed as ECF by the attending clinician. In four cases, the attending clinician was not able to diagnose the cause of death and histology was needed, and in two cases the clinician found a different primary cause of death (one case of trypanosomiasis and one of fasciolosis). The histological data were incredibly valuable when a report was missing. The attending clinicians were good at diagnosing ECF using gross post-mortem findings and preliminary diagnostic tests (blood and lymph smears).

4.4 Discussion

The clinical data collected from the calves was rich and varied. Over 60 different clinical signs were observed, and the clinical parameters showed interesting trends with age.

Generalised lymph node hyperplasia was more commonly associated with young calves. This is a sign commonly associated with the lymphocyte proliferation caused by *Theileria parva* (ECF). Chapter 3 demonstrated that calves were infected at a young age with *T. parva*, and this was likely to have been the cause of the lymph node hyperplasia in young calves (figure 4.15). It could be that this is an artefact. Calves that were sick may have been more likely to be smaller and therefore have smaller lymph node measurements even though their lymph nodes were enlarged when compared to body weight. This may be a more accurate way to measure lymph node hyperplasia.

ECF often leads to a panleukopaenia (Coetzer and Tustin 2004). The mean white blood cell count in sick calves was consistently lower than in healthy calves, also a likely consequence of clinical ECF in the cohort. However, the mean white blood cell counts in the healthy and sick calves diverged at weeks 46 and 51. This may have been due to a cause other than ECF, but it could be that older calves with ECF experienced a greater decrease in white blood cell count, possibly because they survived longer following infection.

Soiling and lethargy were more common in older clinical episodes, and there was a trend for a lower PCV and total serum protein in the older sick calves compared with their healthy counterparts. Inappetence and weight loss were also common in older clinical episodes. Haemonchosis was a significant cause of death in the cohort, and these clinical signs, which are associated with haemonchosis, and parasitic gastroenteritis more generally, suggest that these diseases were common causes of clinical disease, especially in older calves.

Multivariate analysis identified groups of clinical signs that were known to be components of the clinical syndromes for the three main causes of death, and by using three components from a mixed data factor analysis, individuals were able to be partially separated by cause of death. However the aim of the chapter was to identify a

clinical syndrome that could be used to define ECF death both sensitively and specifically without the need for further diagnostic testing. Multivariate analysis did not provide evidence for a specific clinical syndrome.

Multivariate analysis revealed a large amount of variation in clinical presentation between individuals with the same diagnoses. There are a few possible reasons for this observed variation. Calves were visited at varying intervals before death. The varying time from final ante-mortem visit to death will have meant that information for calves with the same diagnoses would have been captured at different points in disease progression. Anecdotally, farmers often struggled to describe the clinical history prior to death making this a poor source of information. Finally, the frequency of co-infection in the cohort calves may go some way to explain the wide variation in clinical presentation between calves, even in those that were attributed the same primary cause. It was common for the expert committee to attribute more than one infectious cause to deaths. Also, calves frequently had several infections identified at or just prior to death that the panel believed to be incidental findings. Mixed data factor analysis showed that the individuals that died from both haemonchosis and ECF were placed somewhere between the mean values for the haemonchosis, and the ECF group (figures 4.24 and 4.26).

Amongst all the clinical variation, the expert panel were able to diagnose death in the majority of calves, including 11 of the 19 calves that were missing histological data. Half of those remaining undiagnosed were also missing an attending clinicians post-mortem report. The diagnosis of ECF was made accurately by gross post-mortem, with 14 of 20 cases with interstitial lymphoblastic infiltration being given a tentative diagnosis of ECF at the time of the post-mortem by the attending clinician. However, there is evidence from these analyses that although ECF may well have been the cause of significant pathology in calves that were diagnosed with this cause of death, there were clinical abnormalities present at death that would not usually be associated with ECF. In many cases these abnormalities were not attributed to another cause by the expert panel. This 'filtering of noise' is a common technique in expert decision making (Hutton and Klein 1999). Expert decision making is a complex task, which practitioners themselves often struggle to articulate. Experts rely on pattern recognition and organise and access knowledge in chunks (Hutton and Klein 1999). It may be that

in this case, the task of attributing a definitive aetiological cause distracted experts from components of the clinical presentation not explained by the defined cause. It was not an aim of the expert panel to attribute cause to all clinical abnormalities, but to diagnose the most (and in some cases 2nd most) likely cause of mortality.

Diagnostic support tools aim to formalise the clinical decision making process to increase reproducibility of findings, to help guide the decision process, or to provide diagnoses in situations where no trained clinicians are available. The decision support tool investigated as part of this chapter (Eisler et al. 2007) weighted several clinical signs for a small set of diseases. The tool was developed using expert opinion.

The decision support tool was found to have a specificity of 83%, but a sensitivity of only 59%. The poor sensitivity was mainly due to misdiagnosis of cases as trypanosomiasis, probably because of high prevalence of anaemia in the calves that died. The mean PCV was 21.9% in those calves that died, and more specifically was 20% in the cases of ECF death. Therefore anaemia was commonly associated with mortality, and was also significantly associated with ECF death. ECF is not usually associated with anaemia, and this finding warrants further investigation as it presents a novel finding. The non-specific finding of anaemia should be directed back to an earlier point. There was a large amount of clinical variation in presentation between individuals who died from the same cause, and this may have been due to co-infections that may or may not have been implicated in the death of the calf. However, it would be prudent to consider that anaemia may be a component of the clinical syndrome associated with ECF.

Haemonchosis was a common co-infection, and a significant cause of mortality, and was more likely to be the cause of anaemia in this population than trypanosomiasis. Haemonchosis was not included on the decision support tool card and tool would benefit from its addition. *T. mutans* was identified as a potential cause of anaemia in early calf hood (Conradie Van Wyk 2012; Moll et al. 1984), and was found to be very common in the IDEAL study cohort (chapter 3) and may have contributed to the low PCV% observed in the population.

On balance, the card showed potential for the correct diagnosis of ECF in this population, but would need modification to improve the sensitivity. There are several

characteristics of the format of the diagnostic support tool tested here that may have pre-disposed it to making mis-diagnoses in the IDEAL cohort. Firstly, the card was not designed to diagnose disease in calves, but in all ages. The clinical presentation in young animals can differ from that expected in adults. Secondly, the card always reaches a diagnosis selected from the options on the card. Therefore for several cases in the cohort it was impossible for it to get the right answer. The tool may benefit from a threshold score, below which the diagnosis is concluded to be unknown. This may solve both the problem of misdiagnosis, and may encourage people to seek further advice in more difficult cases when possible.

This card based tool was designed for contempory use at the pen-side. It offers a guide to inexperienced personnel on the clinical signs to look for (Eisler et al. 2012). However, diagnostic support tools are likely to be more successful if based on an electronic platform such as a mobile phone. This has already been explored by Mckendrick et al. (2000) who developed an online tool called CaDDiS. This format allows more diseases to be incorporated and would allow the integration of uncertainty.

The expert panel formed for investigation of the IDEAL cohort deaths is assumed throughout to have made correct diagnoses. However, it must be considered that in some cases the decision support tool may have been correct and the panel incorrect, or that in some cases both methods made a misdiagnosis.

In conclusion, it was not possible to identify a set of clinical signs, or syndrome, that always identified cases of ECF, and excluded other causes. In keeping with current knowledge of the clinical syndrome associated with ECF, lymph node hyperplasia and pyrexia were found to be significantly associated with the disease. However, anaemia was also significantly associated with the ECF, although it a was common sign generally in the cohort. It is most likely that this pathology was due to a common co-occuring pathogen. Expert panel decision was not able to be matched on accuracy by any of the methods investigated here. A relief, no doubt, to all clinicians.

Chapter 5

Clinical episodes in the cohort: their distribution and characteristics

Chapter abstract

This chapter describes the occurrence of ill health within the cohort, and investigates the concept of a sickly calf. It describes the outcome *'clinical episode'* which was used to classify whether the calf was ill at each visit. It was found that a large number of calves passed through their first year of life without clinical disease being observed, and that a few calves experienced the majority of clinical episodes. Multiple clinical episodes were apparently related in time, suggesting that they were due either to the same or connected pathogenic processes. Low birth weight, older farmers, and larger herds were significantly associated with clinical disease. *T. parva* and heavy gastrointestinal nematode infestations were associated with clinical disease and death in the cohort, but these diseases were not perceived by farmers to be causing an impact in their cattle. It is suggested that education and awareness programmes would allow farmers to make more informed choices about the management of their calves and reduce potential losses from clinical disease.

5.1 Introduction

Morbidity was one of the key outcomes measured in the cohort. *Theileria parva*, the potential cause of ECF, was observed to be very common, and to cause a large number of deaths in the cohort (chapter 3). However, the clinical outcomes observed in calves were very variable, with a suggestion that co-infection was leading to indistinct syndromes (chapter 4). Previous research has generally concentrated on specific causes of morbidity (Gitau et al. (1999); Muraguri et al. (2005); Swai et al. (2009), and reviewed by (Phiri et al. 2010)), but this may not be appropriate in this environment, where animals are often co-infected with several potential pathogens at once, and all may be contributing to ill health. It may be more suitable to investigate exposures whose modification would act to reduce all-cause burden of disease.

Disease in production animals is assumed to be costly, but the real impact of livestock disease is poorly understood (Perry and Grace 2009). The costs associated with periods of ill-health can be postulated to result from decreased productivity, and increased investment of time, feed, and services (professional veterinary advice and pharmaceuticals) (Perry and Grace 2009). Periods of ill health should also be considered to impact on animal welfare. A better understanding of the factors that may increase or decrease the likelihood of developing poor health will allow stake-holders to encourage evidence based behaviour change and direct resources more effectively to reduce the burden of disease.

This chapter, based on the description of clinical episodes in the cohort, seeks to define and identify risk factors for being a sickly calf.

5.2 Materials and Methods

5.2.1 Clinical episodes in the routine and extra clinical visits

Clinical episode was defined according to methods described in chapter 2, section 2.2.4.

All routine visits that were classified as to whether they were a clinical episode or not were included in the analysis, as were all post-mortem visits of either infectious or unknown cause, but not the eight post-mortem visits made to calves that died of non-infectious causes. There were seven routine visits that were followed on the same day by calf death. In these cases the information from the two visits was amalgamated and both visits treated as a single clinical episode. These amalgamated visits were identified as post-mortem visits. It would not have been correct to have considered these as two separate incidents of disease, especially as for some calves the post-mortem was a result of euthanasia at the time of the routine visit. The inclusion of both visits would have led to double counting of clinical episodes.

Clinical episode visits identified outside of routine visits that were not associated with a post-mortem were not included in analyses. This includes the six calves whose deaths were not observed.

5.2.2 Ill health and the use of veterinary treatments in herds

At the recruitment visit, the farmer was asked to report the diseases or syndromes that caused problems in the cattle herd on the farm at the present time. They were asked to rank the diseases or syndromes into first, second, and third place. Subsequently, at each routine visit farmers were requested to report any diseases or syndromes that had been observed in, and any treatments that had been given to the herd and / or the dam in the five weeks up to each routine visit.

5.2.3 The number of clinical episodes per calf

The number of clinical episodes per calf was investigated using distributions that describe count data. This was particularly appropriate in this context as the data were highly skewed and non-normal (Du et al. 2011), meaning that linear models would have been innappropriate. Three different distributions were investigated for their fit to the observed data.

The binomial distribution describes event data, where the outcome is either present or

absent. It investigates the probability of an event at a given trial assuming that the result of each trial is independent of all others (Thrusfield 2005).

The poisson distribution is often used to model rare events. Its key characteristic is that the mean and variance are equal. It models count data where events are independent of each other in either space or time (Thrusfield 2005; Dohoo et al. 2009). If count data deviates from a poisson distribution it indicates a departure from randomness. The key parameter in a poisson distribution is λ, the average count per unit time / area.

Over dispersion is common in count data, and violates the assumption of a poisson distribution. A negative binomial distribution allows the variance to be larger than the mean (Dohoo et al. 2009), and is a good measure of aggregation (Poulin 2008). Formally, it is the probability of observing y failures before the rth success. Negative binomial distributions are commonly used to describe the distributions of parasites in hosts, where the distribution is characterised by a small proportion of potential hosts being infected by a large proportion of the parasite population (May and Woolhouse 1993; Grafen and Woolhouse 1993). The measure of aggregation is $\kappa = \frac{\bar{x}}{s^2 - \bar{x}^2}$, where $\bar{x}$ = sample mean, and s^2 = sample variance (Gregory and Woolhouse 1993). As κ approaches infinity, the distribution approaches a poisson distribution, and events are randomly distributed between hosts.

A Pearson's χ^2 test was used as a goodness of fit test to investigate the difference between the observed count data and that predicted by the distributions described above (Dohoo et al. 2009).

5.2.4 Investigating risk factors associated with being a sick calf

Calves were defined as being a sick calf if they had at least one clinical episode (including death) during their time in the study. Logistic regression modelling was used to investigate exposures associated with the odds of a calf being a sick calf. Only time independent variables were able to be investigated. Univariable screening of biologically plausible exposures and selection of a final model was carried out as described in section 2.4.2 in chapter 2

5.2.5 Investigating the association of specific pathogens with clinical episodes

For investigation of the association between potential pathogens and ill health at a population level, time discrete hazard analysis (TDHA) was used to describe the proportional hazard by week of a calf's first clinical episode depending on exposure or parasite burden (see chapter 2, section 2.4.2). This was limited to those infections for which there was diagnostic data at all routine visits. An assumption of the model was that the change in the hazard from exposure to the infections was proportional across all ages. It is likely that this was not the case, but there were too few events at each time point to allow investigation of the effect of an interaction between age and infection on hazard of clinical episode.

Seroconversion was used to define exposure to the tick borne diseases (chapter 2, section 2.3.2). The seroconversion data from the next visit was associated with the current visit, because seroconversion was likely to be observed following the development of clinical signs. Only visits at week 1 to 41 were able to be assessed as it was not possible for a calf to seroconvert at week 51 (chapter 2, section 2.3.2).

Parasitaemia was included using the semi-quantitative measure recorded from inspection of blood smears (chapter 2, section 2.3.1).

Strongyle egg count data were collected from examination of faecal material (chapter 2, section 2.3.1). The strongyle eggs counts from faecal examination were not normally distributed. The data were transformed using the natural log (see figure 5.1). Following this transform the distribution was bimodal and it was necessary to categorise it for inclusion in models.

A log transformation was also applied to the tropical livestock units (TLU) data, which was also not normally distributed. A large number of farms had few animals, and so a low value for TLU.

Some variables included in the multivariable logistic regression model had missing data. The affect of this on coefficient estimates was investigated using multiple imputation methods (King et al. 2001) using the *Amelia* package (Honaker et al. 2011) in R (R Development Core Team 2010). Missing data were assumed to be missing at

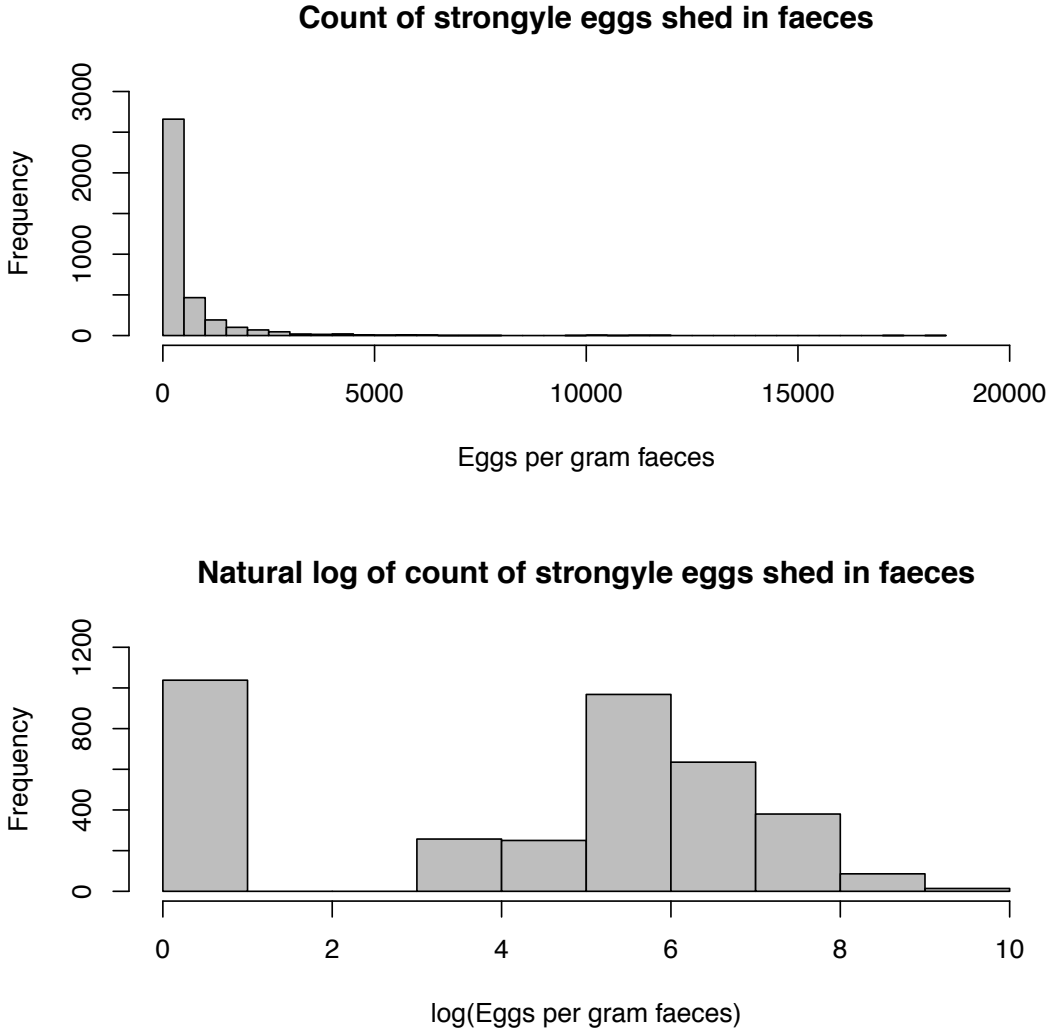

Figure 5.1: The distribution of numbers of strongyle eggs shed in faeces and the distribution once that count was logged.

random. *Amelia* uses the covariance pattern of variables within other individuals to predict the distribution of each of the missing values. A range of predictions are added to a series of models and each of these models can be compared. This allows the effect of the missing data and the association of the variable with the outcome to be investigated.

5.3 Results

5.3.1 Clinical episodes in the routine and extra clinical visits

During the three years of data collection, there were 548 recruitment visits made, 4334 routine 5-weekly visits, 82 post-mortem visits, 455 final visits made at one year (after which the calf was dropped), and 216 clinical visits made outside of the 5-weekly routine visit regime. In addition, there were weekly visits to calves by local animal health assistants (LAHA) in each of the sublocations. These visits often triggered the clinical visits that were conducted outside of the routine visits, but the visits themselves were not recorded in the database. Each of the routine visits and extra clinical visits were classified as to whether they were clinical episodes or not. 179 of the 216 extra clinical visits were classified as a clinical episode following assessment by the IDEAL team, and 307 of the routine visits were classified as clinical episodes. LAHAs visited the farms four times for every one time the IDEAL teams visited. Therefore it would have been expected that they would have detected four times the number of clinical episodes, but it can be seen from the numbers above that this was not the case. This is assumed to have been under-reporting by the LAHAs rather than over-reporting by the IDEAL teams.

As well as the low number of reports, there were different rates of reporting of clinical episodes by LAHAs by sublocation, and these did not match the rate of reporting by the IDEAL teams. The ratio of LAHA clinical episodes to IDEAL detected clinical episodes ranged from 1.4 to 0.08. The LAHAs were required to record that they had visited and this was checked at each 5-weekly IDEAL visit. They were also required to report to one of the IDEAL team with their findings. However, it is likely that some

LAHAs were more diligent, or more observant, or more sensitive to clinical disease than others.

For this reason it was decided not to include the extra-routine clinical visits in analyses investigating trends at a population level.

5325 routine visits were included in the analysis here. The analyses also include 74 post-mortem visits, which included those where the cause of death was either infectious or unknown, and incorporated information from the seven routine visits that led immediately to a post-mortem visit. The eight post-mortem visits following non-infectious deaths were not included. The six calves that died unobserved were censored from their last routine visit.

5.3.2 Ill health and the use of veterinary treatments in herds

Farmers were asked at the recruitment visit, to report which cattle diseases or symptoms often occurred on their farm. A total of 427 farms reported at least one problem on their farm (78%). On 221 farms, disease was reported to be a problem, but the calf had no ill health reported during its time in the study.

Trypanosomiasis was perceived as a significant problem, ranked the most important disease by 161 farmers, and the second most important disease by 25 farmers. Tick borne diseases were ranked as most important on 34 farms, and the second most important on 19 farms. Helminthiasis was ranked as either the most important disease or second most important disease by 20 farmers. East coast fever was reported to be a top three problem on nine farms. Some farmers were unable to identify specific infectious causes of ill health and instead described syndromes that affected their herds. Diarrheic disorders were reported to be one of the top three problems in adult cattle on 29 farms, and in calves on 13 farms. On 31 farms, the clinical sign ‘decreased appetite’ was reported to be the most significant problem. A total of 44 different diseases, syndromes, or clinical signs were reported to affect herds. Calf mortality was only reported to be a significant problem by one farmer.

Current health problems were reported in study cattle herds in 330 of the 5399 routine and post-mortem visits (6%). These reports came from 201 of the 548 farms (37%). Of

these farms, many reported only one incidence of ill health (100 farms). However, 56 farms reported two incidences, and two farms reported problems in the herd at every visit made to the calf. A total of eight of the reports of ill health in the herd happened in the same inter-visit period as a post-mortem visit. The vast majority of the incidences of disease affected less than 10% of the herd (272 of 330 visits), and 163 involved the dam of the calf (49%). In 33 visits both the calf and the herd were simultaneously suffering from ill health.

The most commonly reported problem in herds was a rough staring coat (117 visits), and the second most common, weight loss or loss of condition (84 visits). A decreased appetite was reported in 35 visits.

Treatments were reported to have been given to cohort herds in 1540 of the 5399 5-week inter-visit periods (29%). In 1213 of these visits, the farmer did not know what at least one of the products was that had been used. There were 303 reports of use of the antibiotic, oxytetracycline, and 405 reports of use of the insecticide, amitraz. Use of the anthelminthic, levamisole was reported in 40 visits and albendazole in 77. Five farmers reported having used traditional herbal remedies.

5.3.3 The number of clinical episodes per calf

A total of 295 calves left the study without experiencing a clinical episode. The mean number of clinical episodes per calf was 0.7. The majority of the remaining calves only experienced a single incident (figure 5.2). Some 6% of routine visits were classified as clinical episodes.

The number of clinical episodes per calf was modelled using a binomial, poisson, and a negative binomial distribution, and the predicted distributions compared to the observed data. The number of clinical episodes in calves was predicted very well by a negative binomial distribution (Pearson's χ-squared test, p-value = 0.942). This was not the case for the prediction from a binomial distribution (Pearson's χ-squared test, p-value = 0.019). The fit of poisson distribution was not significantly different to the observed data (Pearson's χ-squared test, p-value = 0.28), but the fit was not as good as with a negative binomial (figure 5.2). The mean to variance ratio of the observed count

Table 5.1: Counts of clinical episodes (CE) per calf observed in the population, and that predicted by a binomial (binom. dist.), a poisson (poisson dist.), and a negative binomial (neg. binom dist.) distribution.

No. CE per calf	No. calves	No. calves binom. dist.	No. calves poisson dist.	No. calves neg. binom dist.
0	295	248	276	299
1	170	204	189	161
2	59	76	65	61
3	15	17	15	19
4	6	3	3	6
5	2	0	0	2
6	0	0	0	0
7	0	0	0	0
8	1	0	0	0

data were 1.32, and an approximate measure of aggregation, κ, was 2.13. A κ of less than 1 is usually taken to denote highly aggregated data, and more than 5 to denote a random distribution of events (Crawley 2007). Therefore there is evidence here for moderate aggregation of clinical episodes within calves.

There were 253 first clinical episodes. The mean age at first clinical episode (for those calves with at least one) was 140 days. The probability of having at least one clinical episode by one year was 0.51 (95% confidence interval, 0.41-0.6, figure 5.3). There was some correlation between geographical location and the probability of having at least one clinical episode (figure 5.5) with those calves further south and closer to the lake apparently more likely to suffer clinical disease. The hazard of first clinical episode rose sharply following the recruitment visit (figure 5.4) and then stabilised up until week 26. After this point the hazard of a visit being a first clinical episode declined up to one year old with a small increase at week 41.

As well as the 253 first clinical episodes, there were 83 second, 24 third, nine fourth, and three fifth clinical episodes. One calf had eight clinical episodes. The calves that went on to suffer a second clinical episode often did so within five weeks of their first episode (33 at the next routine visit, and 14 at death following a clinical episode in a routine visit). For those calves that had a third clinical episode, this was commonly consecutive to the first and second clinical episode (figures 5.6 and 5.7), although several calves did show a longer gap. This data suggests that many clinical episodes within calves may have been related, even if they did not represent a continual state of

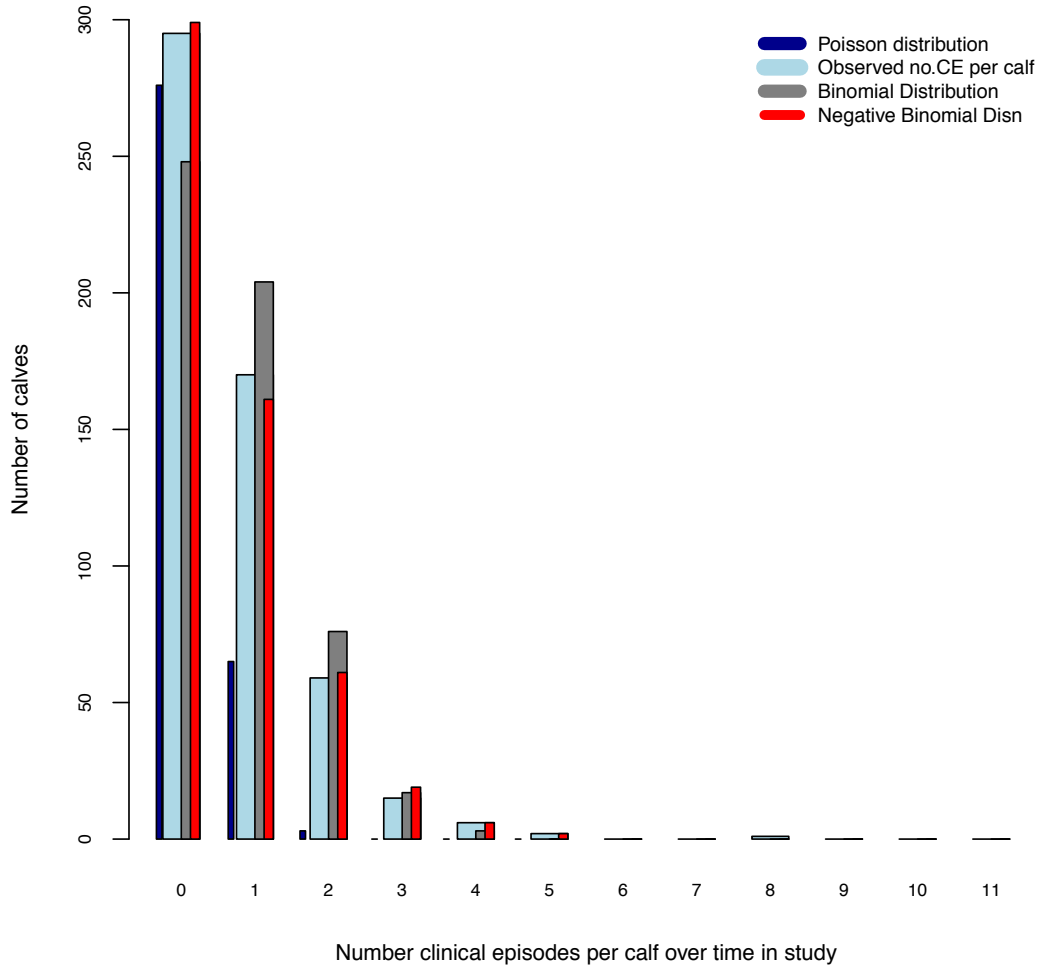

Figure 5.2: Number of clinical episode per calf, and the counts predicted by both a binomial and negative binomial distribution

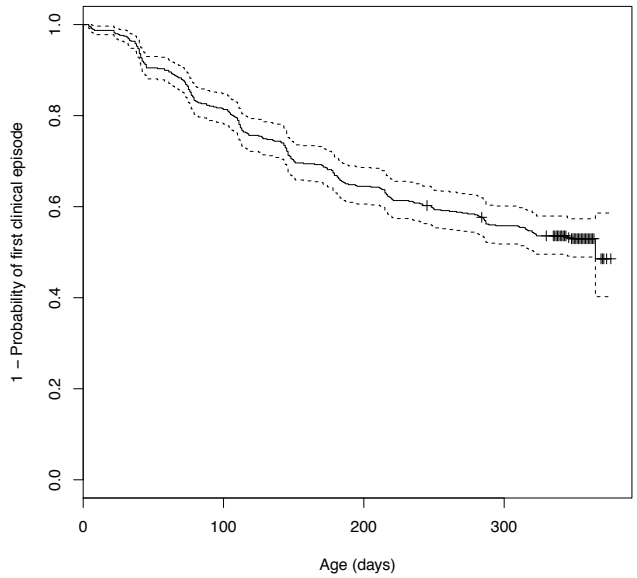

Figure 5.3: Kaplan-Meier curve for probability of first clinical episode.

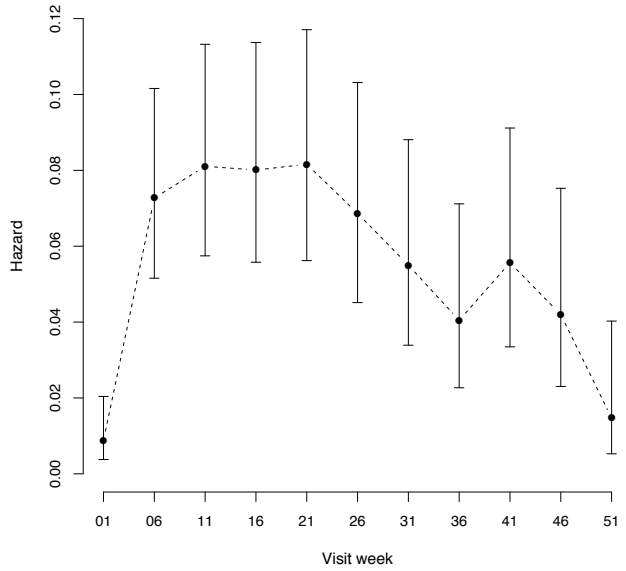

Figure 5.4: Hazard of clinical episode by week

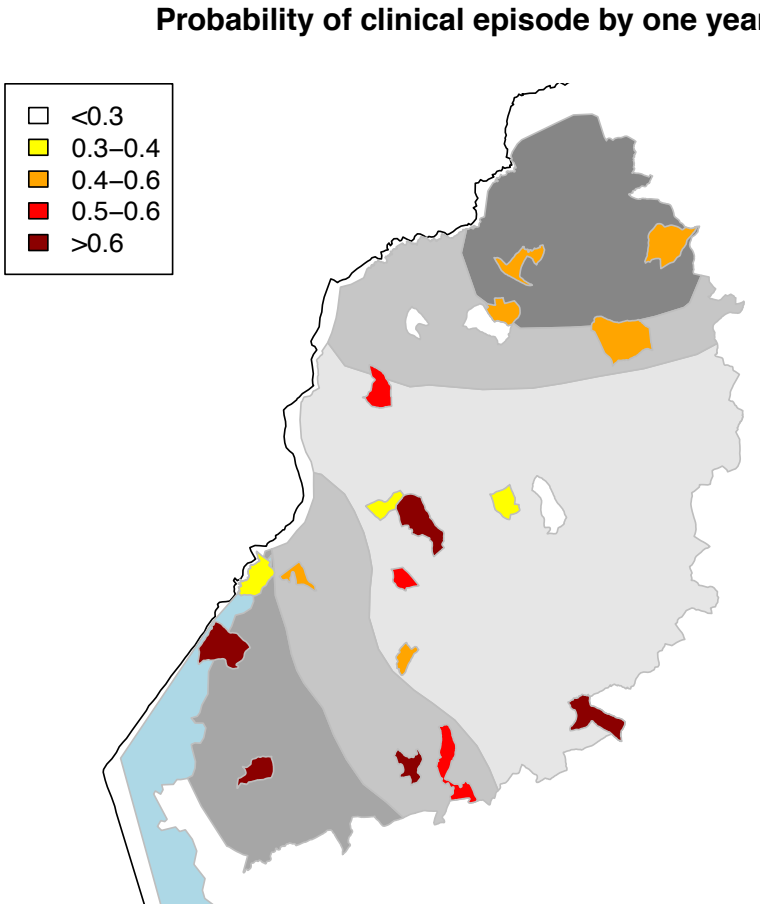

Figure 5.5: The probability of having a clinical episode by 1 year by sublocation. The different zones represent the 5 AEZ, and each coloured region represents a sublocation. For more detail refer to chapter 2, figure 2.2.

ill health that stretched across, in this case, ten weeks. This finding helps to explain figure 5.2 which shows aggregation in the clinical episode count data. The likely cause of aggregation in this data were the consecutive nature of many of the clinical episodes and their likely relatedness.

5.3.4 Exposures associated with being a sickly calf

Calves were classified as sickly if they had at least one clinical episode during their time in the study. Exposures associated with this outcome were investigated for significance and a final multivariable logistic model was developed (table 5.2). This model excluded seven calves due to missing data. The effect of missing data were investigated using imputation. Missing data were found to decrease the coefficient estimate associated with supplementary feeding (associated with a decreased odds of event), so this variable was dropped from the final model.

It was found that calves that had a lower body weight at the recruitment visit, were owned by older farmers, were from larger herds (increase in TLU), or were from dams with high *T. parva* antibody titres were more likely to experience ill health within their first year of life.

Table 5.2: Multivariable logistic regression model for the outcome, clinical episode ever. number of calves included in model = 541. OR = odds ratio, LCL = lower bound of 95% confidence internal, UCL = upper bound of 95% confidence interval.

	Estimate	Std. error	P value	OR	OR_LCL	OR_UCL
(Intercept)	-1.509	0.424	<0.001	0.221	0.096	0.508
ELISA PP *T. parva* Dam	0.010	0.004	0.006	1.010	1.003	1.018
Birth weight <20Kg	-	-	-	-	-	-
Birth weight 20-25Kg	-0.415	0.196	0.034	0.660	0.449	0.970
Birth weight >25Kg	-1.000	0.502	0.046	0.368	0.138	0.984
Farmer age (per 10 years)	0.143	0.066	0.030	1.154	1.014	1.313
log(Tropical livestock units)	0.301	0.142	0.034	1.351	1.023	1.783

5.3.5 Events associated with a clinical episode visit

Clinical episode was a generic term for any ill health. Time discrete hazard analysis was used to investigate which pathogens were significantly associated with the first

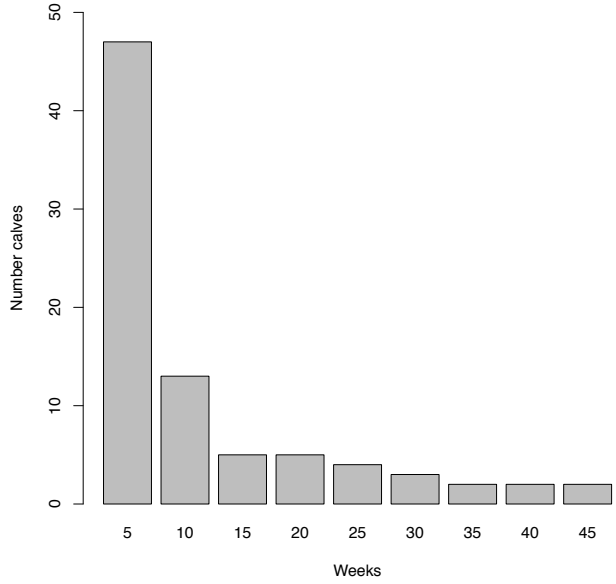

Figure 5.6: The number of weeks between a calf's first and second clinical episode.

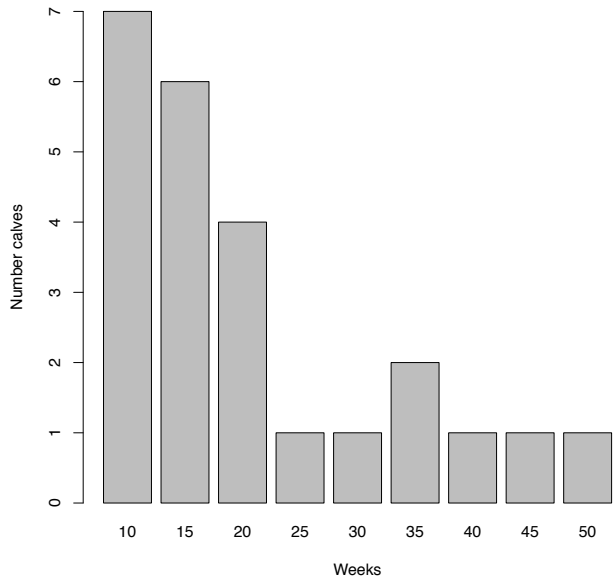

Figure 5.7: The number of weeks between a calf's first and third clinical episode.

clinical episode that calves experienced. Only infections that had been tested for at every visit could be investigated here, so this is not a comprehensive view of all possible potential pathogens. However, it gives an initial idea of which pathogens could be linked to clinical disease in this cohort. An interaction with age at event was not included, and so the model assumes that the infections had a proportional affect on hazard over all time points. All pathogens, for which by-visit data were available, were included. The routine visits at weeks 46 and 51 were not able to be included in models investigating seroconversion because seroconversion was only measured up to week 46. Each pathogen was investigated in a univariable model and these are shown in tables 5.3 to 5.12.

Seroconversion to *T. parva* at the next visit was significantly associated with a visit being a first clinical episode (table 5.3), giving evidence for *T. parva* as a significant cause of clinical disease in the cohort. There was not a significant association between hazard of first clinical episode and seroconversion to *T. mutans*. Identification of *Theileria* spp. piroplasms in blood smears was not significantly associated with clinical disease (table 5.11), most likely because of the large number of infections with *Theileria* spp. such as *T. mutans* that are not thought to be pathogenic. However, having a high parasitaemia was associated with clinical disease (table 5.12).

Faecal egg count burden was investigated for its association with the hazard of first clinical episode (figure 5.8 and table 5.9). Having no strongyle eggs was associated with an increased hazard of clinical episode when compared to calves that were shedding 400 to 2980 strongyle eggs per gram of faeces at the visit of interest ($exponential(6)$ to $exponential(8)$). Shedding more than 2980 strongyle eggs per gram of faeces was significantly associated with an increased hazard of a visit being a clinical episode. The differences in hazard between the first four groups were not significant, so the strongyle egg counts were incorporated into a maximal model as only two groups of low and high strongyle eggs per gram of faeces (threshold of 2980 strongyle eggs ($>$ or $<$ $exponential(8)$)

There was no evidence from this analysis that *A. marginale*, *B. bigemina*, *Trypanosoma* species, or the presence of *R. appendiculatus* were significant causes of clinical disease at a population level. However, there was a statistically significant increase in hazard

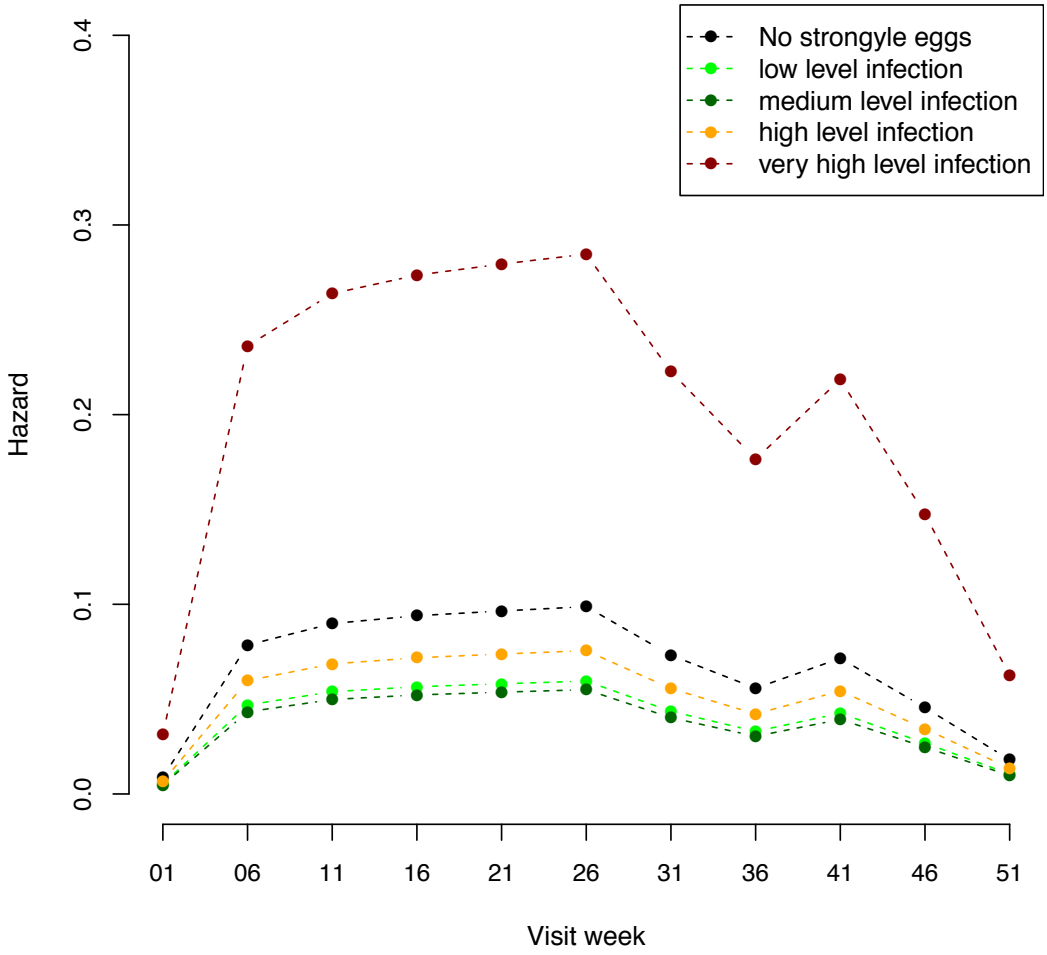

Figure 5.8: Time discrete hazard analysis for hazard of first clinical episode and its association with different levels of strongyle egg shedding in faeces. No eggs = < 7 eggs per gram (EPG), level1 = 7-54 EPG, level2 = 55-403 EPG, level3 = 404-2980 EPG, level 4 = >2980 EPG. This plot shows the hazard of clinical episode by visit associated with each of the strongyle egg count burdens. The hazards for each burden are joined by a dotted line to allow the hazard between groups to be compared more easily. 644 visits were missing data for strongyle egg count.

of first clinical episode if *A. variagatum* was identified at that visit (table 5.8).

5.4 Discussion

Moll et al. (1984) observed every calf recruited to a study in Mara, Kenya, to have experienced morbidity associated with *T. parva* by one year old. However, the levels of morbidity observed by Gitau et al. (1994) were somewhat lower. A total of 26.6% of calves were observed to experience morbidity by 1 year old. However, this study only examined exotic and cross-breed calves. This study only identified calf age to be associated with morbidity, with diarrhoea recorded as the main syndrome. A majority of the calves recruited to the IDEAL cohort left the study at one year old without a single clinical episode being recorded in their routine visits (53%). However, this is likely to be an underestimation of the number of calves affected by clinical episodes. Calves were observed in a snapshot every five weeks. All clinical disease that did not span the observation period will have been missed (apart from mortality). The next largest group of calves only had a single clinical episode. However, it must be noted that all those calves that died suddenly without previous ill health being observed were in this group.

Clinical episodes were found to be moderately aggregated within calves. However, it was found that multiple clinical episodes within calves were often consecutive, and so were possibly related by cause. Whether this represents an increase in clustering of all-cause ill health within calves is questionable. As multiple clinical episodes were so often related to each other it was reasonable to classify calves as ever or never having had a clinical episode, for investigation of risk factors associated with being a sickly calf. However, the investigation of whether being ill once made a calf more susceptible to being ill again was rather difficult in this cohort, because calves that died were obviously unable to accumulate further episodes.

There were four exposures identified as significantly associated with being a sickly calf. These were low birth weight, older farmers, increased herd size, and higher *T. parva* antibody titres in the dams. Both the herd size and the dam antibody titre were likely to have been correlated with infection pressure. It is likely that a larger number

of animals would have led to an increased infection pressure inside the homestead, both from increased contamination and also a greater number of contacts with animals from other herds whilst out grazing. The vast majority of calves received colostrum from their dams, but for *T. parva* this antibody is not believed to offer any protection against clinical disease, a finding supported here.

It is possible that frail older farmers were less able to tend to their animals or were less observant leading to reduced support for animals showing the first signs of ill health. It is also possible that older farmers had access to lower quality grazing, especially as it is common in this region for family owned land to become increasingly divided between members of the family as sons mature and marry. However, further investigation would be needed to establish whether the reasons for this observed effect can be further broken down. This would require collection of further data.

The finding of a connection between birth weight and future health was interesting. Launching an immune response against an infectious disease requires large amounts of energy, and combined with the appetite suppression associated with sepsis can send animals into a state of negative energy balance (Lochmiller and Deerenberg 2000). Calves in the study were frequently infected with potential pathogens at a very young age (chapter 3 demonstrated the young age at which many calves became infected with the potentially pathogenic *T. parva*). Calves born larger with more energy reserves were likely to withstand the energy demands of an activated immune system for longer periods. An ability to maintain a more effective and sustained immune response may have reduced the pathogenic effects of infections. Low birth weight is commonly identified as a risk factor for disease in later life in human infants (Aylward et al. 1989; Conley et al. 2003; Oreopoulos et al. 2008). However, these sorts of studies often struggle to separate the real effect of low birth weight and the affects of the factors that correlate with low birth weight such as poor maternal nutrition, which may be linked to poverty and poorer living conditions. In the context of the IDEAL calves, it is possible that those dams that gave birth to smaller calves were of poorer condition and would have consequently produced lower quality colostrum. However, body condition of the dam at birth was not found to be associated with clinical disease. It may be that those dams in low body condition were in that state because of a genetic potential to invest in foetal growth to the detriment of their own condition. If low birth weight calves are

more susceptible to ill health then it may be advantageous for farmers to invest resources, either by supplementary feeding or provision of preventative drug application, in these small calves in the hope of reducing losses due to ill health. However, if small birth weight was actually correlated with another problem exerting a direct effect on clinical disease then such interventions would not be successful.

Analysis showed clinical disease due to *T. parva* was possibly burden dependent as a high piroplasm parasitaemia, rather than just the presence of piroplasms, was significantly associated with clinical disease. However, this finding may simply suggest that the peak of parasitaemia coincides with clinical signs. An alternative hypothesis is that the high parasitaemia observed associated with clinical disease may have been a recrudescence of previous infections with other *Theileria* species. This can occur when animals previously infected with other species haemoparasites become stressed by a period of ill heath. The stress allows quiescent infections to re-establish (McHardy and Kiara 1995). Moll et al. (1984) suggested that this effect may also have been causing a drop in PCV observed to be associated with *T. parva* infection.

East Coast fever (ECF) and haemonchosis were found to be significant causes of both mortality (chapters 3 and 4) and morbidity. Interestingly, although we observed East Coast fever to be the most significant cause of death in calves (chapter 3, section 3.3.8, and chapter 4, section 4.3.3), this disease was not identified by IDEAL farmers to be important (see section 5.3.2). This shows a mismatch between the perception and reality of disease threats. The top three causes of mortality identified during IDEAL were not commonly perceived as important by farmers. The reasons for this mismatch are complex, but part of the explanation may be the difficulty people often have with assessing actual as opposed to perceived risk (Perry and Grace 2009). Farmers also tend to over-estimate the impact of diseases with overt and distinctive clinical signs, or those that are highly publicised, and tend to under estimate those with chronic onset or those that affect lower value animals (Perry and Grace 2009).

The perceived risk of trypanosomiasis in the region is likely to be connected with the high profile Farming in Tsetse Controlled Areas (FITCA 2005) project that took place in Western Province from 1999 until 2004. This project carried out extensive tsetse trapping and community education and awareness campaigns in areas included in the

IDEAL study site. Education and awareness programmes were likely to have increased perceived importance, and may have raised awareness of the risk to human health associated with the rarer species of *Trypanosoma* species circulating in this region. Also, this project reported a reduction in tsetse numbers of 95%, so the mismatch may be as a result of an old threat that has been well managed by intervention in this region.

The lack of awareness of the impact of *T. parva* may point to an ambivalence towards East Coast Fever in the face of so little progress in prevention. There has been little change in impact from that seen by Barnett (1957) 50 years ago. The losses incurred from *T. parva* may be accepted as natural loss; an inevitability of keeping stock in this region. It also could be that calves are viewed as having comparatively low value when compared with other members of the herd, as young calves have yet to receive substantial investment. However, it must be noted that the most commonly used drug (when farmers knew the drug that had been used) was a tick control product, amitraz, that is not effective against tsetse. It is not known whether the farmers were using the drug in response to, or to prevent losses associated with tick-borne diseases.

Anthelminthics were also used by several farmers, again not a disease that was perceived to be important in cattle herds.

It appears that the treatments farmers were using had little correlation with what they believed were the threats on their farms, but had a reasonable correlation with the actual threats (or at least those in calves). People's perception of the impact of diseases is often not correlated with actual impact (Perry and Grace 2009). This highlights that farmers in the region would benefit greatly from training and education in the actual disease threats facing their herds and how to recognise them, the losses that these diseases may cause, and the treatments and preventative measures available to reduce those losses.

Table 5.3: Time discrete hazard analysis of the association between *T. parva* infection and first clinical episode occurrence. number of calves = 544

	Estimate	Std. Error	P value	Base haz	CI 2.5% hazard	CI 97.5% hazard	Hazard *T. parva* +ve
week 1	-4.51	0.42	<0.001	0.011	0.005	0.024	0.032
week 6	-2.67	0.20	<0.001	0.065	0.045	0.093	0.172
week 11	-2.64	0.20	<0.001	0.066	0.046	0.095	0.175
week 16	-2.85	0.22	<0.001	0.055	0.036	0.081	0.147
week 21	-2.79	0.22	<0.001	0.058	0.039	0.086	0.155
week 26	-2.94	0.24	<0.001	0.05	0.032	0.078	0.136
week 31	-3.21	0.28	<0.001	0.039	0.023	0.065	0.108
week 36	-3.48	0.32	<0.001	0.03	0.016	0.055	0.084
week 41	-3.12	0.29	<0.001	0.042	0.025	0.072	0.116
T. parva +ve	1.09	0.19	<0.001	-	-	-	-

Table 5.4: Time discrete hazard analysis of the association between *T. mutans* infection and first clinical episode occurrence. number of calves = 544

	Estimate	Std. Error	P value	Base haz	CI 2.5% hazard	CI 97.5% hazard	Hazard *T. mutans* +ve
week 1	-5.46	0.51	<0.001	0.00	0.002	0.012	0.006
week 6	-3.41	0.24	<0.001	0.03	0.02	0.05	0.043
week 11	-3.14	0.23	<0.001	0.04	0.027	0.064	0.055
week 16	-3.11	0.24	<0.001	0.04	0.027	0.067	0.057
week 21	-2.92	0.24	<0.001	0.05	0.032	0.079	0.068
week 26	-3.03	0.28	<0.001	0.05	0.027	0.076	0.061
week 31	-3.23	0.32	<0.001	0.04	0.02	0.069	0.051
week 36	-3.44	0.38	<0.001	0.03	0.015	0.063	0.042
week 41	-3.11	0.34	<0.001	0.04	0.023	0.08	0.057
T. mutans +ve	0.30	0.28	0.28	-	-	-	-

Table 5.5: Time discrete hazard analysis of the association between *A. marginale* infection and first clinical episode occurrence. number of calves = 544

	Estimate	Std. Error	P value	Base haz	CI 2.5% hazard	CI 97.5% hazard	Hazard *A. marginale* +ve
week 1	-5.43	0.51	<0.001	0.004	0.002	0.012	0.006
week 6	-3.35	0.23	<0.001	0.034	0.022	0.052	0.046
week 11	-3.11	0.23	<0.001	0.043	0.028	0.065	0.058
week 16	-3.11	0.24	<0.001	0.043	0.027	0.067	0.058
week 21	-2.93	0.24	<0.001	0.051	0.032	0.079	0.069
week 26	-3.04	0.28	<0.001	0.046	0.027	0.076	0.062
week 31	-3.25	0.33	<0.001	0.037	0.02	0.069	0.051
week 36	-3.45	0.38	<0.001	0.031	0.015	0.062	0.042
week 41	-3.12	0.34	<0.001	0.042	0.022	0.079	0.058
A. marginale +ve	0.33	0.39	0.41	-	-	-	-

Table 5.6: Time discrete hazard analysis of the association between *B. bigemina* infection and first clinical episode occurrence. number of calves = 544

	Estimate	Std. Error	P value	Base haz	CI 2.5% hazard	CI 97.5% hazard	Hazard *B. bigemina* +ve
week 1	-5.45	0.51	<0.001	0.004	0.002	0.012	0.002
week 6	-3.35	0.23	<0.001	0.034	0.022	0.052	0.019
week 11	-3.10	0.23	<0.001	0.043	0.028	0.065	0.024
week 16	-3.08	0.24	<0.001	0.044	0.028	0.069	0.025
week 21	-2.89	0.24	<0.001	0.053	0.033	0.082	0.03
week 26	-3.02	0.28	<0.001	0.047	0.028	0.078	0.026
week 31	-3.21	0.32	<0.001	0.039	0.021	0.071	0.022
week 36	-3.41	0.38	<0.001	0.032	0.015	0.064	0.018
week 41	-3.08	0.34	<0.001	0.044	0.023	0.082	0.025
B. bigemina +ve	-0.60	0.72	0.41	-	-	-	-

Table 5.7: Time discrete hazard analysis of the association between *R. appendiculatus* infection and first clinical episode occurrence. number of calves = 544

	Estimate	Std. Error	P value	Base haz	CI 2.5% hazard	CI 97.5% hazard	*R. appendiculatus* +ve
week 1	-4.51	0.47	<0.001	0.011	0.004	0.027	0.007
week 6	-2.22	0.28	<0.001	0.098	0.059	0.159	0.068
week 11	-2.14	0.28	<0.001	0.105	0.063	0.171	0.073
week 16	-2.16	0.30	<0.001	0.104	0.06	0.172	0.072
week 21	-2.07	0.30	<0.001	0.112	0.065	0.186	0.078
week 26	-2.24	0.32	<0.001	0.096	0.054	0.167	0.067
week 31	-2.47	0.35	<0.001	0.078	0.041	0.143	0.054
week 36	-2.78	0.38	<0.001	0.058	0.028	0.116	0.04
week 41	-2.44	0.36	<0.001	0.08	0.041	0.15	0.055
week 46	-2.82	0.40	<0.001	0.056	0.026	0.116	0.038
week 51	-3.81	0.58	<0.001	0.022	0.007	0.065	0.015
R. appendiculatus +ve	-0.40	0.24	0.10	-	-	-	-

Table 5.8: Time discrete hazard analysis of the association between *A. variagatum* infection and first clinical episode occurrence. number of calves = 544

	Estimate	Std. Error	P value	Base haz	CI 2.5% hazard	CI 97.5% hazard	*A. variagatum* +ve
week 1	-4.58	0.42	<0.001	0.01	0.004	0.023	0.016
week 6	-2.46	0.18	<0.001	0.079	0.057	0.108	0.118
week 11	-2.46	0.19	<0.001	0.079	0.056	0.11	0.119
week 16	-2.56	0.20	<0.001	0.072	0.049	0.103	0.108
week 21	-2.57	0.21	<0.001	0.071	0.049	0.103	0.108
week 26	-2.82	0.23	<0.001	0.056	0.036	0.086	0.085
week 31	-3.10	0.27	<0.001	0.043	0.026	0.071	0.066
week 36	-3.49	0.31	<0.001	0.03	0.016	0.054	0.046
week 41	-3.16	0.28	<0.001	0.041	0.024	0.069	0.062
week 46	-3.60	0.34	<0.001	0.027	0.014	0.051	0.041
week 51	-4.62	0.53	<0.001	0.01	0.004	0.027	0.015
A. variagatum +ve	0.45	0.15	<0.001	-	-	-	-

Table 5.9: Time discrete hazard analysis of the association between Strongyle faecal worm egg infection level and first clinical episode occurrence. number of calves = 544. No eggs = < 7 eggs per gram (EPG) and was the reference level, Strongyle inf lev 1 = 7-54 EPG, Strongyle inf lev 2 = 55-403 EPG, Strongyle inf lev 3 = 404-2980 EPG, Strongyle inf lev 4 = >2980 EPG.

	Estimate	Std. Error	P value	Base haz	CI 2.5% haz	CI 97.5% haz	Haz lev 1	Haz lev 2	Haz lev 3	Haz lev 4
week 1	-4.72	0.49	<0.001	0.009	0.003	0.023	0.005	0.005	0.007	0.031
week 6	-2.46	0.21	<0.001	0.079	0.053	0.114	0.047	0.043	0.06	0.236
week 11	-2.32	0.24	<0.001	0.09	0.058	0.137	0.054	0.05	0.069	0.264
week 16	-2.27	0.28	<0.001	0.094	0.057	0.151	0.057	0.052	0.072	0.274
week 21	-2.24	0.28	<0.001	0.096	0.058	0.156	0.058	0.054	0.074	0.279
week 26	-2.21	0.30	<0.001	0.099	0.058	0.164	0.06	0.055	0.076	0.285
week 31	-2.54	0.34	<0.001	0.073	0.039	0.134	0.044	0.04	0.056	0.223
week 36	-2.83	0.37	<0.001	0.056	0.028	0.109	0.033	0.03	0.042	0.177
week 41	-2.56	0.34	<0.001	0.072	0.038	0.131	0.043	0.039	0.054	0.219
week 46	-3.04	0.41	<0.001	0.045	0.021	0.096	0.027	0.025	0.034	0.148
week 51	-4.00	0.65	<0.001	0.018	0.005	0.061	0.01	0.01	0.014	0.062
Strongyle inf lev 1	-0.55	0.34	0.11	-	-	-	-	-	-	-
Strongyle inf lev 2	-0.63	0.23	0.01	-	-	-	-	-	-	-
Strongyle inf lev 3	-0.29	0.23	0.20	-	-	-	-	-	-	-
Strongyle inf lev 4	1.29	0.32	<0.001	-	-	-	-	-	-	-

Table 5.10: Time discrete hazard analysis of the association between *Trypanosoma* infection and first clinical episode occurrence. number of calves = 544

	Estimate	Std. Error	P value	Base haz	CI 2.5% hazard	CI 97.5% hazard	*Trypanosoma* +ve
week 1	-4.73	0.43	<0.001	0.009	0.004	0.02	0.012
week 6	-2.53	0.18	<0.001	0.074	0.053	0.103	0.096
week 11	-2.43	0.19	<0.001	0.081	0.057	0.113	0.105
week 16	-2.45	0.20	<0.001	0.079	0.055	0.113	0.103
week 21	-2.43	0.20	<0.001	0.081	0.056	0.116	0.105
week 26	-2.61	0.23	<0.001	0.068	0.045	0.103	0.089
week 31	-2.85	0.26	<0.001	0.055	0.034	0.088	0.072
week 36	-3.17	0.30	<0.001	0.04	0.023	0.071	0.053
week 41	-2.83	0.27	<0.001	0.056	0.034	0.092	0.073
week 46	-3.22	0.33	<0.001	0.038	0.021	0.071	0.051
week 51	-4.20	0.53	<0.001	0.015	0.005	0.04	0.02
Trypanosoma +ve	0.29	0.51	0.57	-	-	-	-

Table 5.11: Time discrete hazard analysis of the association between a calf having a *Theileria* +ve blood smear and first clinical episode occurrence. number of calves = 544

	Estimate	Std. Error	P value	Base haz	CI 2.5% hazard	CI 97.5% hazard	Haz blood smear *Theileria* +ve
week 1	-4.92	0.45	0.00	0.007	0.003	0.017	0.008
week 6	-2.82	0.20	0.00	0.056	0.039	0.081	0.058
week 11	-2.76	0.21	0.00	0.059	0.04	0.088	0.061
week 16	-2.71	0.23	0.00	0.062	0.041	0.094	0.064
week 21	-2.65	0.24	0.00	0.066	0.042	0.101	0.068
week 26	-2.77	0.26	0.00	0.059	0.036	0.095	0.061
week 31	-3.02	0.30	0.00	0.047	0.027	0.081	0.048
week 36	-3.29	0.35	0.00	0.036	0.018	0.068	0.037
week 41	-2.92	0.31	0.00	0.051	0.028	0.09	0.053
week 46	-3.24	0.36	0.00	0.038	0.019	0.074	0.039
week 51	-5.61	1.09	0.00	0.004	0	0.03	0.004
Blood smear *Theileria* +ve	0.04	0.16	0.83	-	-	-	-

Table 5.12: Time discrete hazard analysis of the association between the level of *Theileria* infection on blood smear and first clinical episode occurrence. number of calves = 544

	Estimate	Std. Error	Pr(>|z|)	Base haz	CI2.5_haz	CI97.5_haz	HazardLev1	HazardLev2	HazardLev3
week 1	-4.79	0.44	0.00	0.008	0.004	0.019	0.009	0.03	0.269
week 6	-2.83	0.20	0.00	0.056	0.038	0.081	0.06	0.182	0.723
week 11	-2.67	0.20	0.00	0.065	0.045	0.094	0.069	0.207	0.754
week 16	-2.72	0.22	0.00	0.062	0.041	0.092	0.066	0.198	0.744
week 21	-2.62	0.22	0.00	0.068	0.045	0.101	0.072	0.214	0.762
week 26	-2.81	0.25	0.00	0.057	0.036	0.089	0.061	0.184	0.726
week 31	-3.09	0.28	0.00	0.044	0.025	0.073	0.047	0.146	0.667
week 36	-3.34	0.33	0.00	0.034	0.018	0.063	0.037	0.117	0.609
week 41	-3.04	0.30	0.00	0.046	0.026	0.079	0.049	0.152	0.677
week 46	-3.34	0.35	0.00	0.034	0.018	0.065	0.037	0.118	0.61
week 51	-4.69	0.63	0.00	0.009	0.003	0.031	0.01	0.033	0.288
Theileria infection level 1	0.07	0.16	0.66	-	-	-	-	-	-
Theileria infection level 2	1.32	0.28	0.00	-	-	-	-	-	-
Theileria infection level 3	3.79	0.63	0.00	-	-	-	-	-	-

Chapter 6

Risk factors for East Coast fever death following infection with *Theileria parva*

Chapter abstract

Host response to infection can vary. The variation in expression is affected by the host, parasite, and environmental factors, and also the host's previous and concomitant infections. The aim of this work was to describe the exposures experienced by animals that led to either death or survival following infection with *T. parva*. The clinical syndrome associated with the infection is referred to as East Coast fever (ECF). 71% of the cohort were infected with *T. parva* in their first year of life, but only a fraction (8.7%) went on to die from that infection. Unmatched and matched nested case control study designs were used to investigate the risk factors associated with death following *Theileria parva* infection (ECF death) in these calves.

6.1 Introduction

Host outcome following infection is known to vary, but identifying the reasons for that variation can be challenging, especially in natural populations where several risk factors interact. This web of interactions is further complicated by concomitant or previous exposure to the same or additional species of infectious agent.

The presence of *T. parva* acts as a major constraint on the introduction of more productive exotic breeds of cattle to east Africa (Kivaria et al. 2007), and also continues to cause substantial production losses in indigenous breed cattle (Barnett 1957; Moll et al. 1984; Gitau et al. 1999; Gachohi et al. 2010). Activation of cytotoxic T cells is believed to be required for the bovine immune system to successfully control this infection, a response that is not only parasite species specific but is also strain specific (Taracha et al. 1995). Therefore, recovery from a first infection with *T. parva* may not prevent clinical disease or death following a subsequent infection with a different strain type. This highly specific immune response and the widespread distribution of the tick has made the disease very difficult to control.

Initial analysis of data from the study showed very high rates of exposure to *T. parva*, but a relatively small proportion of ECF deaths. This led to the key question, what factors are related to death following infection with *T. parva*?

6.2 Materials and Methods

Causes of death were defined according to methods in section 2.2.5 in chapter 2. An ECF death was a calf that died from ECF as either the primary or contributing cause of death. In calves that survived their infection, seroconversion to *T. parva* was used to identify infection with the parasite. Seroconversion was defined using a moving window rule that used ELISA results from three consecutive routine visits to measure a rising and sustained antibody titre, and was carried out according to methods in section 2.3.2 in chapter 2. On comparison with veterinary opinion, this was found to be a highly specific method, and so would ensure a high certainty that infection had occurred.

To investigate risk factors associated with death following infection with *T. parva*, a nested case-control design was used. Cases were defined as those calves that died from ECF before they seroconverted to *T. parva*. Calves that died from ECF following seroconversion to *T. parva* were excluded, as it was not possible to know whether they died from a recrudescence of their first infection or from a super-infection. As the interest of this work was host outcome following infection, controls were those calves that seroconverted to *T. parva* before leaving the study. Due to the difficulty of diagnosis of some deaths, all calves that died before one year old from all other known and unknown causes were excluded from the control group. This was to avoid unidentified cases entering the control group. The two groups of calves described formed an unmatched case-control data set and this was analysed using mixed effects logistic regression using the package *lme4* (Bates and Maechler 2010) in *R* (R Development Core Team 2010). A random effect for sublocation was included to account for the clustering by sublocation caused by the stratified study design.

An age at first infection with *T. parva* was calculated for all case and control calves. For those calves that died, this was defined as the week of the next routine visit the calf would have received had death not occurred. For example, a calf that died between visit weeks six and eleven would have been defined as having been exposed to *T. parva* at week eleven. For the calves that seroconverted to *T. parva* the age at seroconversion was the second visit of the three used to calculate whether seroconversion occurred (chapter 2, section 2.3.2). Therefore, the time of infection in both cases and controls was the first visit in which *T. parva* was detected.

Age was identified as a risk factor for death, and therefore an age matched nested case control design was used to control for age and so allow incorporation of time dependent variables to models. Calves were matched in groups by their age at exposure to *T. parva*. The matched case-control study was analysed using conditional logistic regression (Dohoo et al. 2009) using the *survival* package (Therneau and Lumley 2010) in *R*.

The form of the conditional logistic model is $logit(Y) = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + ... + \beta_i X_i + \gamma_i + \epsilon_i$ where γ is the random covariate or the panel indicator (matched groups in the conditional logistic model), and ϵ is the error term.

Two different methods of matching were used. The first used all the available controls and used the week of infection with *T. parva* to identify sets of cases and controls (panel indicator). For this analysis, some sets contained more than one case. As this was a retrospective study, all available controls were included in the analysis. Although little power is gained from the inclusion of more than four controls to every case (Dohoo et al. 2009), it was thought that this would reduce the introduction of any bias from selection of controls. A second and more traditional matching method employed matching three controls to each case. Three controls were randomly assigned to each case in turn by week of *T. parva* infection. Each set of four calves were given a set identification number. This gave a data set containing 96 calves in 24 sets. The set identification number was used as the panel indicator.

A list of biologically plausible risk factors was compiled for association with ECF death. These are listed in appendix J, section J.1, table J.3. *T. mutans*, *Anaplasma marginale*, and *Babesia bigemina* infections were detected using seroconversion. The seroconversion rule used for identification of controls was not suitable for the identification of exposure in this context. For calves that died by visit week 11, their exposure status could not have been established as they did not have the required three visits. Also, for calves that were infected at later visits, controls had an extra visit in which serum was collected compared to cases. Calves that died did not have serum collected from their visit at death. The last point that it would have been possible for cases to seroconvert according to the three point rule would have been ten weeks (two routine visits) before the visit of death. Therefore, for the investigation of the association of co-infections with ECF death, a less stringent rule was applied that only required ELISA results from two consecutive routine visits. Exposure was defined to be present when a rising titre of at least the cut-off was recorded between two visits. The cut-off for *T. mutans* was 20 PP, and for *B. bigemina* and *A. marginale* was 15 PP. This method allowed the incorporation of the maximum number of cases and controls to be included in models investigating the association of prior exposure to tick borne diseases measured using ELISA. Cases that died before their routine visit at week six were not able to be included because they did not have the required two serum samples to measure a rising titre. Cases that died between weeks 46 and 51 were not able to be included, because controls were not able to seroconvert at week 51. Therefore, the

matched case control study contained all cases and control calves that were exposed to *T. parva* at weeks 11 to 46 inclusive. Although the two sample rule for detecting seroconversion was possibly less specific that the method requiring three samples, any misclassification would have been as likely in cases as in controls. Therefore, this method would not have introduced bias to model coefficient estimates. None of the exposures detected using seroconversion were included in the unmatched case control study, because this would have required the exclusion of all cases that died before their routine visit at week six, and therefore also all the controls that seroconverted at week six. It was decided that for the unmatched model it would be more suitable to incorporate the maximum number of cases, and use the age matched case control study to investigate exposures associated with prior infection.

Calves were defined as having been exposed to *T. mutans*, *A. marginale*, or *B. bigemina* before *T. parva* infection if they had a rising titre of at least 5PP at any point before the week of *T. parva* infection. For controls this was the week of seroconversion. For the cases this was the week associated with ECF death.

Trypanosoma infections were identified by microscopy. Three microscopy techniques were applied to samples from every routine visit to identify *Trypanosoma* spp. These are described in Nantulya (1990) and involved examination of both blood smears and buffy coat. Any infection detected up to the point of infection with *T. parva* identified a calf as having had a prior infection of *Trypanosoma* spp..

Strongyle egg counts and *Haemonchus* egg counts included in models were those counts taken from the faecal samples collected at the visit before *T. parva* infection (2, section 2.3.1). The egg counts were log transformed and then categorised as described in chapter 5, section 5.2.

Throughout the text where *"prior infection with"* is used, it refers to the above definitions.

The amount of European taurine genetic introgression was calculated using analysis of single nucleotide polymorphisms (SNPs) from the Illumina© Bovine SNP50 beadchip. 45,000 SNPs were randomly selected from across the genome. The extent of European taurine admixture was investigated using a Bayesian clustering method implemented in the STRUCTURE program (Pritchard et al. 2000) as described in Ndila (2012). The

level of European breed genetic introgression in the cohort was generally low, but some calves had more recent European breed ancestry. The level was categorised into low or moderate to high (cut-off = 0.0156%). This categorisation suggests the last European ancestor to have been six or fewer generations ago. The mean % European introgression in the cohort was 0.02% with a minimum of 0.0003% and a maximum of 0.34%.

Univariable screening of all exposures was carried out and model building and selection carried out according to methods in chapter 2, sections 2.4.2, 2.4.3, and 2.4.5. Interactions between the significant variables in the final model and other screened risk factors were investigated in turn.

6.3 Results

6.3.1 The ECF deaths

Thirty-four calves died from ECF, two of these as the secondary cause to heartwater (*Ehrilichia ruminatum*) or black quarter (clostridial infection). Of the 34 cases of ECF death, 28 died before seroconversion to *T. parva*. Six calves died at various times following seroconversion to *T. parva* (figure 6.1). It is not known whether these calves died from a recrudescence of their first infection, or from a subsequent super-infection.

Of the 548 calves recruited to the study, 362 calves seroconverted to *T. parva* including the six calves that seroconverted to *T. parva* prior to ECF death (66%). A total of 390 calves were infected with *T. parva* in the first year of life. This comprised 362 calves that seroconverted to *T. parva* and 28 for which *T. parva* infection was first detected at post-mortem examination (figure 6.2). A total of 340 calves seroconverted to *T. parva* and survived that infection, living beyond their final visit at 1 year old.

Investigation of the proportion of infections with *T. parva* that led to death by age suggests that infections in young calves were more likely to lead to ECF death (figure 6.3). The proportion of infections leading to death declined up to week 26 and then remained fairly stable at around 0.05% of infections. There was a slight rise at week

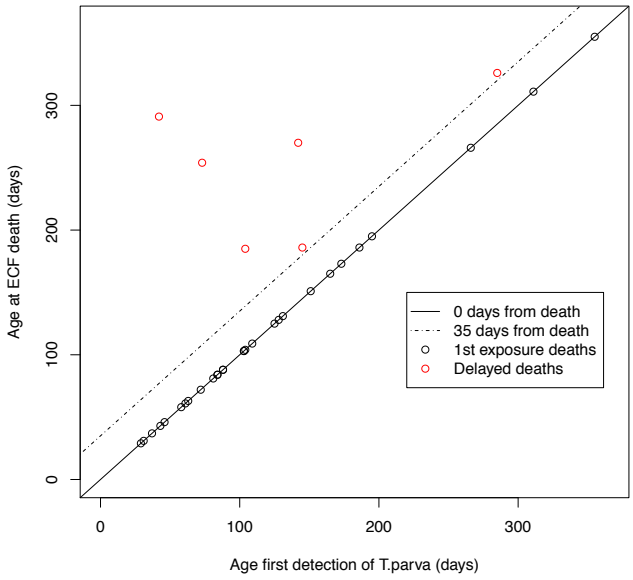

Figure 6.1: The age at first infection with *T. parva* and the age at ECF death

41. However, the number of deaths each week was small leading to wide confidence intervals. For incorporation to the unmatched case control model, age at exposure was grouped into three categories, weeks 1 and 6, weeks 16 and 21, and weeks 26 to 51 inclusive. The calves that suffered delayed ECF death initially seroconverted to *T. parva* at weeks 6, 11, 16, and two at week 21.

A similar analysis, but investigating agroecological zone (AEZ) suggested that the proportion of infections leading to death across all zones was similar, except for in lower middle three (AEZ 5 (LM3)), where 15% of infections led to death (figure 6.4). There were three delayed deaths in AEZ 5, one in AEZ 4, and two in AEZ 3.

Both body weight at recruitment and farmer age were identified as risk factors for being a sick calf (chapter 5, section 5.3.4). As *T. parva* was a significant cause of morbidity, it was hypothesised that these risk factors may also have been associated with ECF death .

The proportion of *T. parva* infections leading to ECF death was at a minimum for those farmers aged between 40 and 50 years old (figure 6.5). There was a general increase in

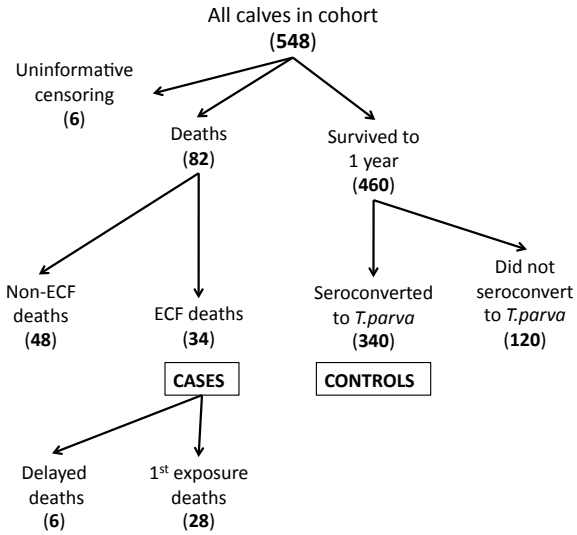

Figure 6.2: Flow diagram for selection of cases and controls for unmatched case control study. Controls for the matched case control studies were selected from the 340 unmatched control calves.

the proportion of infections leading to death for calves owned by farmers over 50 years old. The delayed deaths were distributed evenly across all farmer age groups.

There was no apparent association with weight at the recruitment visit (taken as a proxy for birth weight) and the proportion of infections leading to death (figure 6.6). However, none of the 15 calves that were more than 25Kg at birth and were also infected with *T. parva* went on to die from their infection.

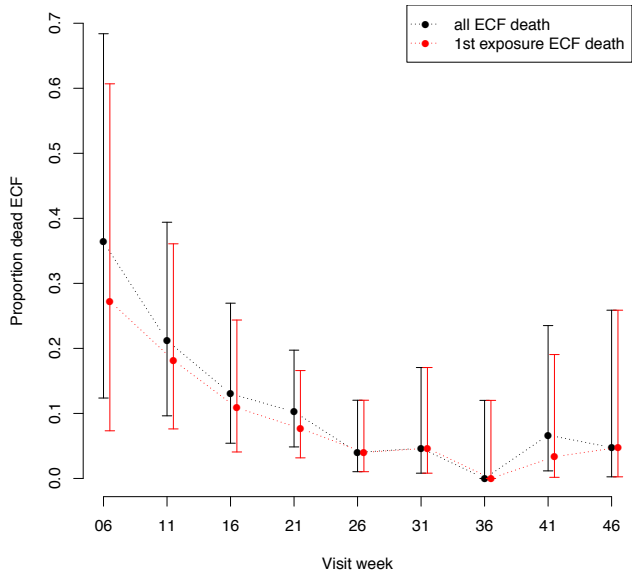

Figure 6.3: The proportion of calves that died from ECF by the age they were first infected with *T. parva*

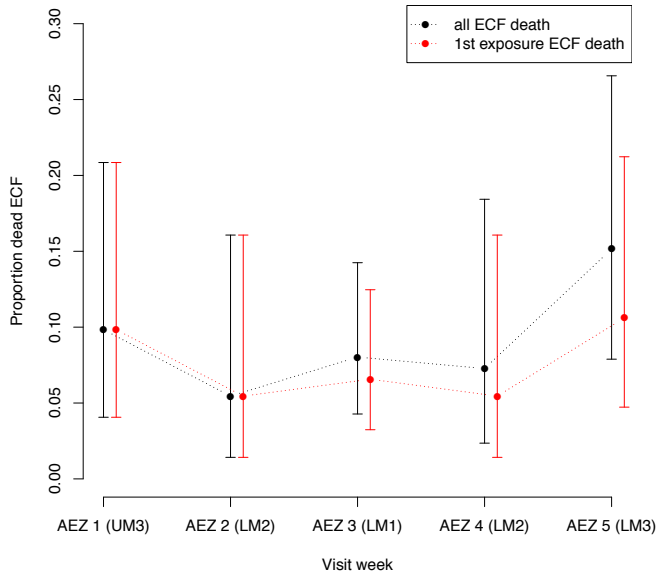

Figure 6.4: The proportion of calves that died from their *T. parva* infection by AEZ

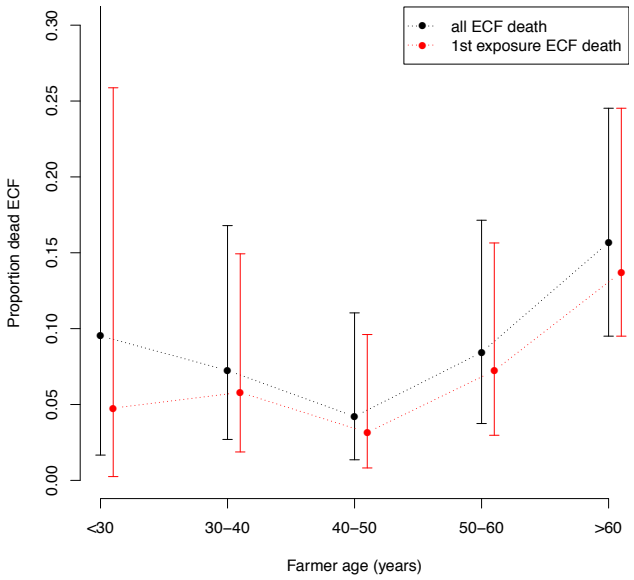

Figure 6.5: The proportion of calves that died from their *T. parva* infection by the age of the farmer.

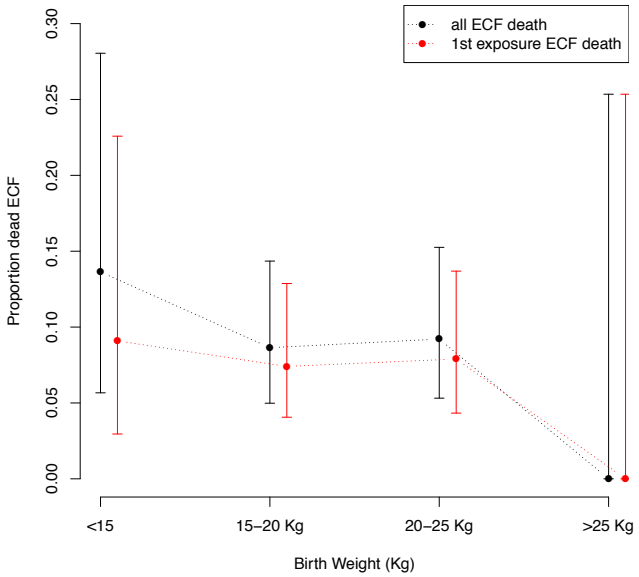

Figure 6.6: The proportion of calves that died from their *T. parva* infection by the calves' body weight at their recruitment visit.

6.3.2 Risk factors for ECF death

Unmatched case control study

The full list of variables investigated for association with ECF death is in appendix J, section J.1. This also contains the unmatched cases control model coefficient estimates from the univariable screening of these variables.

The summary of the parsimonious model is in table 6.1. Calves that were older at infection, and those that were going out grazing with the adults by the time of *T. parva* infection were all at reduced odds of ECF death. There were 27 calves that were missing data for whether they were going out grazing or not by the time of infection with *T. parva*. Only one of these died from ECF.

Farmer age was also a significantly associated risk factor. Calves that were owned by older farmers had a higher odds of ECF death. Farmer age was included as a continuous variable. it did not violate the assumption of linearity in the logit. This was assessed using the Box-Tidwell test.

Table 6.1: Summary table for unmatched case control study. OR = odds ratio. LCL and UCL are the lower and upper limits of a 95% confidence interval.

	Estimate	OR	LCL OR	UCL OR	P value
(Intercept)	-3.139	0.043	0.006	0.309	0.002
Age *T. parva* infection 6-11 weeks	-	-	-	-	-
Age *T. parva* infection 16-21 weeks	-0.947	0.388	0.134	1.122	0.081
Age *T. parva* infection 26-51 weeks	-1.406	0.245	0.079	0.758	0.015
Farmer age (10 years)	0.423	1.526	1.111	2.097	0.009
Grazing FALSE	-	-	-	-	-
Grazing TRUE	-1.994	0.136	0.038	0.482	0.002

Matched case control study

Due to the reduced odds of death in older calves identified by the unmatched case control study and the possible confounding effects of this, cases were matched to controls by age at infection. The two matching methods described in section 6.2 were applied in turn to the data to investigate, particularly, if any time dependent variables were associated with ECF death.

The first analysis included 24 cases and 333 controls. These were all infected with *T. parva* at 11 to 46 weeks. The week of infection with *T. parva* was used as the panel indicator. All time dependent variables including those that were detected using seroconversion were screened for association. The results from univariable screening are in appendix J, section J.2. The summary of the final model is in table 6.2. Farmer age did not significantly contribute to the model. Whether the calf was going out grazing with the adult cattle during the day was associated with a reduced odds of ECF death, and so was robust to matching by age.

Prior exposure to *T. mutans* was significantly associated with a decreased odds of ECF death, and showed a statistically significant interaction with the level of European breed genetic introgression of the calf. Those calves that had high levels of European genetic introgression did not show the reduced odds associated with prior *T. mutans* exposure. When grazing was removed, allowing those calves with missing data into the model, the size of the effect associated with *T. mutans* was reduced from an odds ratio of 0.194 to 0.211 (appendix J, section J.3, table J.4). When prior exposure to *T. mutans* was included in a univariable model the odds associated with the exposure was lower again (0.406) and the variable was no longer significant to the 95% level (p value = 0.063) (appendix J, section J.3, table J.5).

A more traditional balanced matching approach was then applied. The second analysis used sets of one case and three age matched controls. The set identification number was used as the panel indicator. This model incorporated results from 24 cases and 72 controls. The results from univariable screening are in appendix J, section J.1. The final model is in table 6.3. The interaction between prior *T. mutans* exposure and European genetic introgression was no longer significant. Again, grazing was removed from the model to investigate the effect of the addition of calves with missing data for grazing (appendix J, section J.3, table J.6). In this model neither prior exposure to *T. mutans* or its interaction with European genetic introgression were significant at the 95% level. The direction of the effect remained but it was of a slightly lower magnitude. When the non-significant interaction with genetic introgression was removed, the association of *T. mutans* was no longer statistically significant, but the direction of the effect remained (appendix J, section J.3, tables J.7 and J.8).

The relationship between the risk factors grazing, and prior exposure to *T. mutans* and its interaction with European genetic introgression, and the outcome ECF death are shown in two by two tables in appendix J, section J.4

6.4 Discussion

The aim of this work was to identify reasons for the different outcomes following *T. parva* infection observed in this population of calves. Previous published work using experimental infection of cattle has identified three exposures leading to variation in host outcome. A larger infective dose was found to correlate with both decreasing time to onset of pyrexia and time to death (Radley et al. 1974), different strains of *T. parva* have been observed to vary in their pathogenicity (Tindih et al. 2010), and cattle breed is known to affect response to infection, with exotic breeds suffering more severe clinical signs and increased mortality rates (Coetzer and Tustin 2004). Under more natural conditions (not following experimental infection) the responses in the host are likely to be complicated by factors such as levels of nutrition, previous exposure to pathogens, and concomitant infections. The results described here took account of many of the possible confounding factors to allow the effects of co-infection on host response to be observed. However, response to strain and dose were not able to be controlled for.

Infection with *T. parva* at a younger age was identified as a significant risk factor for ECF death. Farmer age was as also identified as a risk factor in the unmatched case control study. However, the effect size was reduced once calf age had been controlled for by matching. It is possible that farmer age was a proxy for an unmeasured variable that was correlated with age at infection. The evidence for this risk factor being associated with ECF death is mixed, but targeted data collection would be needed to investigate whether this observation is repeatable and if so, to try to explain it.

Calves that were going out grazing by the time of infection with *T. parva* were at significantly lower odds of ECF death. Although older calves were more likely to be going out grazing, the association was robust to matching by age. The reasons for the association between access to grazing and a reduced odds of ECF death are not

Table 6.2: Summary of conditional logistic regression model matched by age. The panel indicator used was week of infection. The data set contained 24 cases and 333 controls in eight sets.

	Estimate	OR	LCL OR	UCL OR	P value
T. mutans before *T. parva* FALSE	-	-	-	-	-
T. mutans before *T. parva* TRUE	-1.693	0.184	0.054	0.629	0.007
European taurine genetic introgression FALSE	-	-	-	-	-
European taurine genetic introgression TRUE	-1.526	0.217	0.025	1.859	0.163
Grazing FALSE	-	-	-	-	-
Grazing TRUE	-2.412	0.090	0.019	0.419	0.002
T. mutans before *T. parva* TRUE x European taurine genetic introgression TRUE	3.221	25.050	1.827	343.401	0.016

Table 6.3: Summary of conditional logistic regression model matched age. The panel indicator used was the set identification number.

	Estimate	OR	LCL OR	UCL OR	P value
T. mutans before *T. parva* FALSE	-	-	-	-	-
T. mutans before *T. parva* TRUE	-1.301	0.272	0.076	0.973	0.045
European taurine genetic introgression FALSE	-	-	-	-	-
European taurine genetic introgression TRUE	-0.665	0.514	0.055	4.831	0.561
Grazing FALSE	-	-	-	-	-
Grazing TRUE	-2.090	0.124	0.023	0.666	0.015
T. mutans before *T. parva* TRUE x European taurine genetic introgression TRUE	2.585	13.265	0.871	202.062	0.063

immediately obvious. It would have been expected that calves going out grazing would have been at a greater risk of infection as they were more likely to be exposed to the vector of *T. parva*, *R. appendiculatus*. However, exposure was controlled for by the methods used in these analyses. It could be that being allowed out grazing was correlated with being a calf in good condition as only calves in good condition were allowed out. In turn, calves in good condition at the time of *T. parva* infection may have been more able to resist the pathogenic effects of that infection. It could also be that those calves that were out grazing had access to better fodder, or had more time to suckle from their mother, therefore improving their nutrition, which again may have protected the calf against the pathogenic effects of *T. parva*. As described in chapter 5, mounting an immune response requires a lot of energy. However, whether a calf was still suckling was not found to be significantly associated with ECF death. The reasons for this association remain unclear, and further investigation into correlations with grazing would need to be carried out. Grazing was not a risk factor for *T. mutans* seroconversion, although it could be postulated that calves out grazing were more likely to have been exposed to *T. mutans*.

This study examined a small number of cases of ECF death following infection with *T. parva* and had low statistical power and this limited the ability to find significant effects, and reduced the ability to investigate interactions fully. However, it was able to identify significantly associated risk factors. The study concentrated on the outcome following infection, and this is the likely reason for a lack of association of the risk factors identified in chapter 3. It is likely that the risk factors identified in that chapter determined the age at which animals became infected, and that if infection happened in young calves that infection was more likely to result in death.

This analysis provides equivocal evidence for a protective effect of prior exposure to *T. mutans*. The association of prior exposure to *T. mutans* with a decreased odds of ECF death and its interaction with European breed genetic introgression were robust to matching by age (table 6.2) and the effect direction and significance was not affected by missing data (table J.4). However, prior exposure to *T. mutans* was not significantly associated with ECF death when in a univariable model. When a more traditional case control format was carried out (single cases matched to a balanced number of age matched controls) the direction of the effect remained, but was reduced in size and was

no longer significant at the 95% level. This variable was challenging to incorporate into models due to the means of detection of *T. mutans* exposure. Seroconversion required two samples from consecutive routine visits to observe a rising titre, and this meant that those calves that died before they reached their routine visit at six weeks old could not be investigated for this effect. It was also shown that the risk of ECF death was higher in younger calves. Three cases were not able to be included in the age matched case control analysis because of this, and an additional calf was not able to be included as it died after 46 weeks and therefore had no available age matched controls. The loss of 14% of the cases would have led to a substantial loss of power. The small sample size was a major limitation throughout this analysis limiting the significance of the effects seen.

The identification of prior exposure to *T. mutans* as possibly protective against ECF is interesting and warrants further investigation, even though the results were inconclusive. For *T. parva* the cell mediated response has been shown to be highly specific with limited and unpredictable cross protection even between strains of the same parasite. A high level of antigenic variation in *T. parva* populations is maintained through sexual recombination in the salivary glands of the tick vector (McKeever et al. 1999) causing many different strains of the parasite to co-circulate in a small geograhical area. It has been reported that the strain specific immune response is further complicated by an interaction between *T. parva* strain and the MHC type of the host. This is believed to determine the dominant antigenic response in the host (Goddeeris et al. 1990). Considering this and the differences in the life-cycles of the *T. parva* and *T. mutans*, if there was a protective effect of *T. mutans* as hinted at in this analysis, it is unclear how this protection would be conferred.

The life cycle of *T. mutans* differs from that of *T. parva* in that the main replicative stage occurs not in lymphoblasts but in the erythrocytes. The most common clinical sign associated with the parasite in the cattle host is anaemia and the reduction of red blood cell count is correlated with the number of parasitised erythrocytes (Young et al. 1978). Although there are some reports of clinical disease associated with *T. mutans* (Robson et al. 1977; Young et al. 1978; Saidu et al. 1984; Moll et al. 1984) it is generally believed to be benign. Although the schizont and piroplasm life-stages differ in their prominence between the 2 species, the life cycle is essentially similar and there

are several points at which is could be postulated that the 2 species could interact. The pathogenesis of *T. parva* involves uncontrolled replication of *T. parva* schizont infected lymphoblasts leading to a fatal lymphoproliferative disease. The successful control of this proliferation it thought to depend on a $CD8^+$ T-cell immune response, which is believed to be strain specific (Morrison 2007). There has been very little investigation into the immune response mounted by the host to *T. mutans*.

T. parva shows a certain degree of similarity to human malaria (*Plasmodium spp.*), something proposed by Morrison (2007). The host immune reaction to both *Plasmodium* spp. and *T. parva* are parasite strain specific. Both parasites can lead to repeated episodes of clinical disease in a single host over time, and in both cases this is thought to sometimes be associated with new infections of different strain types. It is the case for both *Theileria* (Bazarusanga et al. 2007) and *Plasmodium* (Maitland et al. 1997) that species of different pathogenicity co-circulate within the same host population. $CD8^+$T cells, as for *T. parva*, are believed to be critical in protective immunity to pre-erythrocytic malaria, but natural killer (NK) cells and interferon γ are also critical to the response (Doolan and Hoffman 1999). NK cells are believed to play an important role in the immune response to protozoan parasites more generally (Korbel et al. 2004).

Plasmodium falciparum and *P. vivax*, both causes of human malaria, have been reported to interact in a similar way to the effect we have observed for *T. mutans* and *T. parva* (Gunewardena et al. 1994; Maitland et al. 1996, 1997). It was found that individuals that were repeatedly infected with *Plasmodium vivax*, the less pathogenic species of malaria, were more resistant to the pathogenic effects of a subsequent *Plasmodium falciparum* infection (Gunewardena et al. 1994; Maitland et al. 1996). Maitland et al. (1997) postulated two means of protection from cross-immunity between malaria parasites; *anti-parasitic* and *anti-toxic* effects (high malaria parasitaemia with little symptomatic disease). The idea that there are similarities between ECF in calves and malaria in humans is not new, but our work encourages further investigation into these similarities.

Another interesting but very preliminary finding of this work was the finding of the interaction between *T. mutans* exposure and the amount of European breed genetic

introgression. We observed that those calves that had more recent European breed ancestors did not appear to benefit from the possible protective effect of *T. mutans*. It is known that European breeds suffer more severe clinical disease and higher incidence of death following *T. parva* infection compared to the East African short horn zebu breed, and so it was hypothesised that calves with a more recent European breed ancestry would have had poorer outcomes following *T. parva* infection. However, the findings here suggest that the relationship may be more complex and would need further investigation. A cattle breed indigenous to India, the Sahiwal, shows tolerance to *T. annulata*. This is a parasite that has many similarities to *T. parva*, but a different geographical range. The relationship between *T. annulata* and both Sahiwal and Holstein-Friesian cattle has been investigated in some detail. Holstein-Friesian cattle suffer from more disease associated with *T. annulata* when compared to local indigenous breeds (Glass et al. 2005). The Sahiwal have been observed to have a much reduced activation of macrophages, the main mechanism of pathogenesis for *T. annulata* (equivalent to *T. parva* activation of lymphocytes) when compared to Holstein-Friesian cattle (Glass and Jensen 2007). This has been shown to be due to an increase in the production of TGF-β2 induced by the parasite in Holstein-Friesian compared to Sahiwal cattle. This increases the invasive potential of *T. annulata* infected macrophages (Chaussepied et al. 2010). Although a different cell type is responsible for the pathogenesis it is possible that a similar mechanism of tolerance occurs in short horn zebu cattle when infected with *T. parva*.

The results from this investigation are inconclusive. There was a significant association and substantial effect of grazing that could not be explained. There was also an affect of age at infection on risk of ECF death, and also equivocal evidence for an association between prior exposure to *T. mutans* and reduced odds of ECF death, and for interaction of this exposure with European breed genetic introgression. The association with *T. mutans* may warrant further investigation, and experimental studies would be the best way to offer evidence for or against these findings. If the initial findings from this work are correct, it is likely that *T. mutans* is influencing the clinical presentation of *T. parva* in endemic regions.

Chapter 7

General Discussion

7.1 The IDEAL project

The IDEAL project was ambitious in its aims, both scientifically and logistically. It successfully recruited and retained a cohort of 548 calves and led to a biobank containing more than 40'000 samples and results from more than 300'000 diagnostic tests. At the start of the project it was anticipated that it would be a calf centred project with no specific pathogen focus. However, as calves began to pass through the study it became apparent that a single pathogen, *T. parva*, was having a significant effect on outcome at both an individual and population level. This finding shaped the focus of this thesis, and throughout the analysis an ever present problem was a lack of power. There was a vast amount of individual variation between the combinations of infections that calves acquired, the order of these infections, and their consequent outcomes. This led to a data set that was fascinating and confusing and that often resembled a set of case studies. Clinical outcome was a major interest of this thesis and even with the high intensity data collection on a large number of calves the intervals between visits were too long to be able to directly compare calves. To fully investigate variation in clinical expression tens of thousands of individuals would have been needed with the full range of disease combinations and the gap between observations would ideally have been one day. However, the results from the IDEAL project and from this thesis have increased the understanding of the effects of infectious disease in

the study population and have highlighted a number of key areas that should be priorities for future work. The stratified design of the project mean that the results are able to be generalised beyond the study population, and the fine detail environmental data allowed many confounders to be controlled for. The project was not designed to suit the aims of just this thesis and had this been the case it may have been designed differently. However, with the broader aims of IDEAL considered the project design was an excellent example and can be built on in future work.

The main theme of this thesis was why did some calves falter and others thrive following infection with *T. parva*? To answer this, there were three key objectives in this thesis. These were to:

- improve understanding of the epidemiology of *T. parva*
- describe variation in host response to infection
- explain variation in host response to infection

For the investigation of these objectives, three outcomes were considered :

- whether the calf became infected or not
- whether the calf experienced a clinical episode or not
- whether the calf died from its first *T. parva* infection or not

7.2 Main findings

Several risk factors were identified that were found to be associated with the three outcomes described above, the hazard of infection with *T. parva*, the odds of experiencing clinical disease, and the odds of ECF mortality following infection. These are summarised in figure 7.1. There are shared risk factors between the different outcomes suggesting that all the outcomes are interconnected. However, the use of a case control design for the final outcome, ECF mortality, controlled for any risk factors associated with becoming infected in the first place.

Higher elevations were significantly associated with a decreased hazard of

seroconversion to *T. parva*. This was most likely correlated with lower temperatures or decreased humidity both of which reduce the survival and reproductive success of the tick *R. appendiculatus* (Norval et al. 1992). Increased herd size was significantly associated with both an increased hazard of seroconversion to *T. parva* and also an increased odds of being a sick calf. This risk factor straddled both outcomes and was again most likely associated with an increase in infection pressure. Both *T. parva* and helminths were significant causes of ill health in the cohort. The larger herds may have been associated with both an increase in risk of *T. parva* infection, and an increase in the burden of helminths on pasture.

Clinical episodes were quite rare in the cohort. Fewer than 50% of calves were observed to suffer one or more clinical episodes during their first year of life. However, this was likely to have been a substantial under-estimate, as clinical disease was only reliably recorded in the 5-weekly routine visits. Only mortality was consistently observed in continuous time. The clinical episodes (and clinical presentation at death) were very varied, even in cases with the same primary cause. Many cases presented with clinical signs that were not linked to either the primary or contributing cause of death. This was highlighted by the large number of cases presenting with anaemia. Although some of the variation in clinical presentation will have been due to variation in the point in the pathogenic process at which calves were observed, it was postulated that some of the clinical sign overlap was due to the contribution of co-infection to the overall clinical presentation. Helminth burden and *T. mutans* were discussed as possible sources of the variation in clinical expression of disease. The amount of variation associated with clinical disease in this cohort raised questions over the effectiveness of clinical diagnosis in this setting. However, it was possible to show that a clinical diagnostic support tool (Eisler et al. 2007) developed for the region had promise, especially for diagnosis of ECF. The analysis and validation of the tool carried out here offers guidance on those parts of the tool that are working well, and those that need further development to allow for its effective use in a real-world context. Also, the work on prevalence of clinical signs with different diseases, and the comparative prevalences of the different causes of mortality and morbidity will help in the further development of such tools. It was found that the tool would benefit from adjustment to local conditions and to age group to allow suitable specificity, and would

greatly benefit from being moved onto a more flexible electronic platform. This is now possible as mobile technology is now so accessible to so many in East Africa.

It is likely that much richer conclusions would have been made about both the presentation of clinical disease and those risk factors predisposing calves to ill health, had clinical disease been more comprehensively monitored. It was not possible as part of this work to attribute cause to clinical episodes, mainly because it was so difficult to identify specific clinical syndromes. Despite these caveats is was possible to identify exposures that increased the risk of ill health, and some interesting observations were made about the clinical presentations in calves.

T. parva was associated with substantial amounts of clinical disease at a population level (chapter 5, section 5.3.5). A total of 6% of the cohort died from East Coast Fever. However, only 8.7% of *T. parva* infections led to death. The losses associated with mortality can be more easily appreciated than those associated with ill health. However, Thumbi (2012) identified a negative association between *T. parva* and growth and this was also observed by Moll et al. (1984), and so it must be considered that, along with losses from mortality, *T. parva* also may have had more insidious detrimental effects on production in the cohort. Ill health due to *T. parva* may well have been associated with reduced growth rates.

Haemonchosis was also identified to have caused a number of mortalities, and helminthiasis more generally was found to have caused significant amounts of clinical disease at a population level. Worm burden was found to be important, as only calves with very high faecal egg counts were shown to suffer clinically from their infection. There was some evidence that a low burden as opposed to having no worms was associated with a reduction in clinical disease (chapter 5, figure 5.8). There is evidence that ill health associated with helminths may have translated to production losses in calves. Heavy helminth burdens were estimated to cause a 3.3% decrease in growth rate per 1000 increase in strongyle egg count per gram of faeces (Thumbi 2012).

It was found that seroconversion to *T. parva* predicted survival following subsequent *T. parva* challenge. Of the 362 calves that seroconverted to *T. parva*, 340 went on to survive beyond 1 year, 16 died from other causes, and 6 calves went on to die from ECF. Therefore, it was shown that, despite the strain specificity of the immune

response to *T. parva*, if a calf survived its first exposure, it was unlikely to succumb to future disease. It has been shown that different strains of *T. parva* have different pathogenicities (Radley et al. 1974), and also that *T. parva* strain and calf MHC type may interact to determine the effectiveness of the immune response against *T. parva* (Morrison 2007). It would therefore be of interest to investigate the both the initial and subsequent strains infecting calves with these different outcomes, and those calves MHC types. It may be possible to observe patterns that would develop the understanding of this interaction between *T. parva* strain and calf genotype.

A low birth weight was a predictor of being a sick calf. This is a complicated phenotype, which is also associated with poor outcomes in human infants. More work would be needed to see whether low birth weight has a direct effect on outcome, or whether low birth weight is correlated with another exposure. Although it might be tempting to advise farmers to offer supplementary feed to low birth weight calves to increase their body weight, it may be that the correction of the low birth weight would have no effect, and that a more distant exposure should be the focus of attention.

It was also found that farmers had a poor understanding of the risk of *T. parva* in their calves. However, there were indications that farmers may have been able to identify sickly calves. Going out grazing was identified as protective against ECF death, and one of the reasons offered for this correlation was that farmers were able to pick out those calves that were healthy and able to withstand the possible rigours of going out grazing. It may be that awareness campaigns would allow farmers to better understand the causes of ill health in their calves, and so be better able to treat and manage cases. This may reduce or prevent losses.

Exposure to *T. mutans* prior to infection with *T. parva* was identified as possibly protective against ECF death in pure East African short horn zebu calves. This finding was equivocal, but could be significant in the epidemiology of *T. parva* and ECF in this region.

T. parva is expected to establish an endemically stable state under conditions such as that in the IDEAL study site (Coetzer and Tustin 2004; Norval et al. 1992). Endemic stability is generally regarded as a steady state where frequent interaction between the host, the parasite, and its vector leads to a high challenge and high levels of immunity

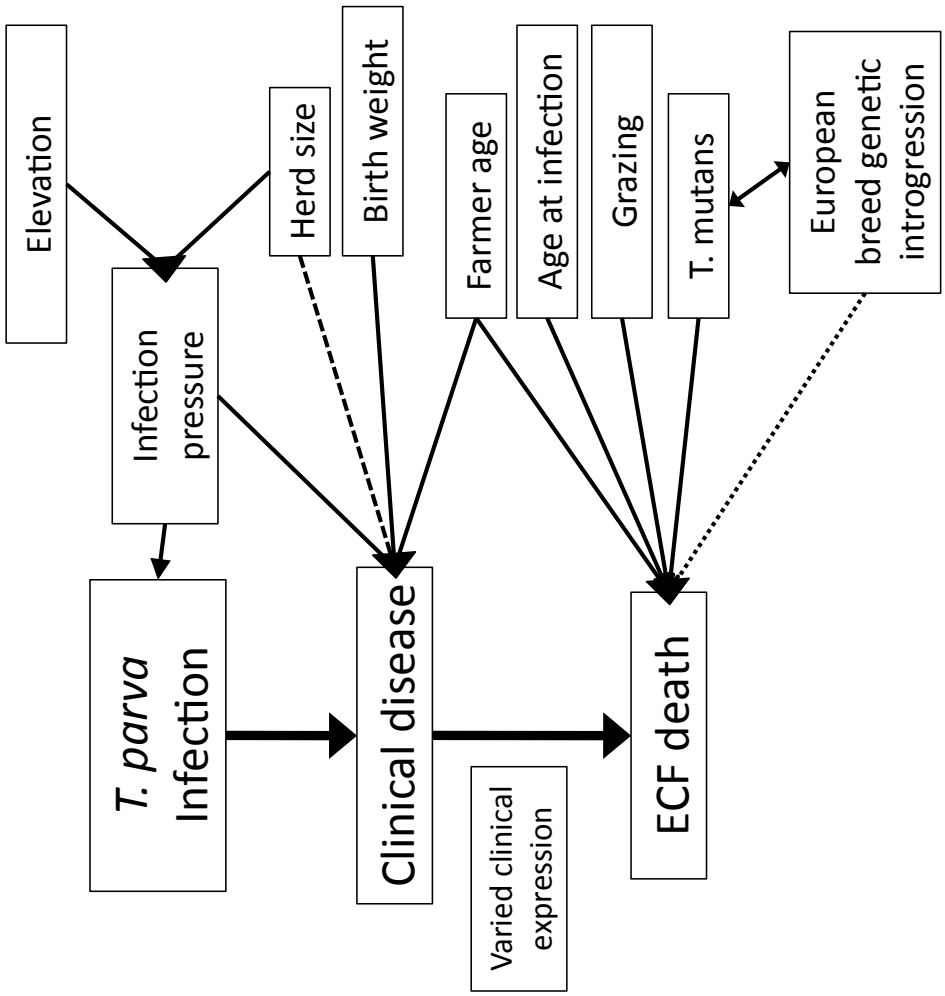

Figure 7.1: A summary of the major findings.

in adult cattle, but a low incidence of clinical disease in both adults and young animals (Jonsson et al. 2012). The majority of the population are expected to become infected by 6 months old, and the levels of morbidity and mortality due to the infection are expected to be constant, and usually low in the population (Norval et al. 1992).

We were able to show that the levels of *T. parva* infection in the cohort were very high, but lower than the 50% 6 month incidence suggested to be needed for endemic stability. The probability of seroconversion to *T. parva* by six months of age was 43.7% (95% confidence interval = 39.2 - 47.9%). Of those calves that were infected with *T. parva*, 8.7% died from their infection. This is very similar to the level observed by Barnett (1957) in the 1950's in Western Kenya. Sublocation and AEZ were not significant predictors of death, and disease did not occur in temporal clusters (figure 7.2). The rate of mortality was apparently constant by age group in the study site for the 3 years of data collection and there was not substantial local variation in risk.

There was variation in hazard by elevation, and by size of herd, but neither of these exposures would have been expected to have led to rapid or seasonal changes in tick numbers. Therefore, whatever time of year a calf was born it was subjected to a similar *T. parva* infection pressure from birth. I believe that *T. parva* was observed to be endemically stable in this region. However, the mechanisms for endemic stability that were developed for *Anaplasma marginale* (anaplasmosis) and *Babesia bovis* (babesiosis) are not suitable for *T. parva*. Calves are protected from anaplasmosis or babesiosis either by maternally derived antibody or innate resistance. These mechanisms give time for young calves to develop acquired immunity to the infections while protected from disease. This study offers evidence that further supports the belief that calves are not protected from *T. parva* by maternal immunity, and that calves are not born with innate resistance to ECF. A different paradigm for endemic stability is required for *T. parva* to that applied to *Babesia* and *Anaplasma*. One possible paradigm was described by Norval et al. (1992):

- There is no seasonal variation anticipated in the population of ticks.
- Carrier animals are common and maintain a low infection rate in ticks.
- The cattle population has a tolerance to *T. parva*.

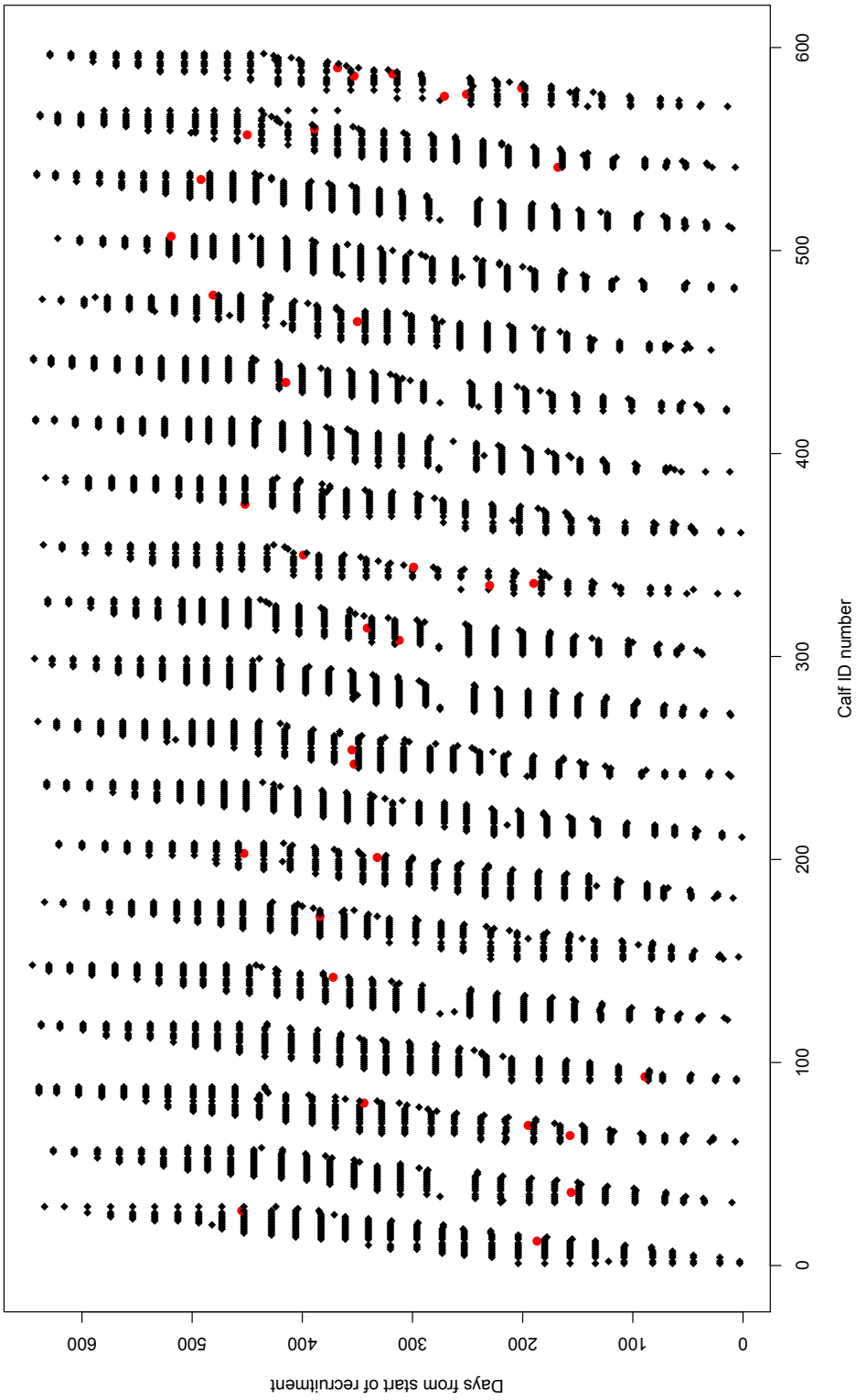

Figure 7.2: The number of days from the start of the project that ECF deaths happened. Normal routine visits or deaths due to other causes are coloured black. Deaths due to ECF are coloured red. The calf ID numbers were clustered by sublocation and ordered by sublocation, so generally low ID numbers are from calves in the north of the study site, and high numbers from the south of the study site.

- Calves are exposed to a small number of sporozoites sufficient to activate the necessary $CD8^+$ cytotoxic T cell response, but below that which would provoke overt clinical disease.
- Calves are exposed to several different *T. parva* strains in this manner to allow a solid immunity to develop.

How do the findings of this thesis fit with current understanding of *T. parva* epidemiology?

The carrier state is believed to be important for maintenance of endemic stability. Evidence from this study suggested that the carrier state was rare. Only 45 calves that had previously seroconverted were RLB positive at their final visit compared to 292 that were RLB negative (chapter 3, table 3.7. Three calves were not tested by RLB at their final visit). However, it was shown by Odongo et al. (2010) that RLB was not an effective method of identifying carrier animals. In carrier animals, *T. parva* is believed to reside in a few schizonts restricted to lymph tissue. Only very few parasite infected cells are released to the circulation making it difficult to detect in blood samples, and more sensitive diagnostic methods are needed. Also, the investigation of calves likely underestimates the prevalence of the carrier state in the cattle population. For a better understanding of the carrier state it will be essential to sample the adult cattle population in the region. Further data collection would be needed to investigate the role of the carrier state in the maintenance of endemic stability in this context, and this would need to be related to the infection rates and intensities in the tick vectors. This data are not available from the IDEAL project.

Tolerance of the host to *T. parva* was also an important part of the endemic stability paradigm. Short horn zebu cattle do have a tolerance to *T. parva*, demonstrated well in this study where over 70% of calves seroconverted to the parasite, but only 8.7% died from their infection, far below the level expected in susceptible breeds such as the Holstein-Friesian where mortality can reach 100% (Coetzer and Tustin 2004; Norval et al. 1992). However, this thesis identified that the relationship between tolerance to ECF and indigenous breeds may be more complicated, and may sometimes involve an interaction with *T. mutans*. The IDEAL project excluded exotic breed cattle from recruitment. However, some calves had small amounts of European *Bos taurus* taurus

ancestry. Although there was no direct association between this and survival or clinical disease, there was some evidence that those calves that had moderate levels of European breed genetic introgression did not benefit from the protective effect against ECF death of *T. mutans* possibly experienced by the pure breed East African short horn zebu calves.

T. mutans, against what was previously thought, has been shown to be far from a benign bystander in the dynamics of disease in the short horn zebu cattle population of Western Province. Apart from the possible protective effect of the parasite, it was identified as contributing to lowered PCV in young calves (Conradie Van Wyk, 2012), and when present with *T. parva* it was seen to act in interaction to reduce growth rate in calves more than the additive effects of the two parasites (Thumbi 2012). Therefore, there is a real need to better understand the epidemiology of *T. mutans*. For this, the diagnostic tools currently available will need further validation. This study identified that the sensitivity of the *T. mutans* ELISA may be low, especially some time after initial infection. It was postulated that this may be due to immune complexes, or because of the use of an IgG_1 specific anti-bovine antibody in the conjugate. An investgation of the effect of immune complexes and IgG isotype on the ELISA performance should be a priority (chapter 3). The prevalence and burden of infection of *T. mutans* in *A. variegatum*, and the population density of *A. variegatum*, and its relationship with calves should be investigated to help better understand transmission. There was also a mismatch identified between infestation of calves with *A. variegatum* and *T. mutans* seroconversion in those calves. It would be beneficial to confirm that *A. variegatum* is the only vector of *T. mutans* in the field. Finally, it will be crucial to experimentally test the relationship between prior infection with *T. mutans*, and survival following infection with *T. parva*.

It has recently been suggested that endemic stability may not be a useful paradigm to discuss the epidemiology of *T. parva* (Jonsson et al. 2012), where the contributions of innate and passive immunity are poorly understood and the requirements for maintenance of the state over time are not suitably defined. However, rather than describing endemic stability as a defunct paradigm, I believe this should offer further motivation to investigate how endemic stability is maintained for *T. parva*. This will allow more effective and safe implementation of control strategies for ECF. The

paradigm for endemic stability may need to include the presence and prevalence of *T. mutans* and its vector.

7.3 Mitigation of the impacts of *T. parva*

Disease due to *T. parva* was not perceived to be a problem by the farmers recruited to the IDEAL study. Even fewer believed calf mortality to be a substantial problem. However, the losses associated with *T. parva* in the cohort were not inconsequential. On farms that keep very few breeding dams, and in a system where the presence of a calf is believed to be essential for milk let down and the calving interval is long, the death of a calf leads to a substantial loss (Rege et al. 2001). Although the proportion of animals that suffered disease compared to the number infected was low, the impact of *T. parva* on the East African short horn zebu population of calves was considerable, with effects observed on both growth (Thumbi 2012), and mortality rates.

However, endemic stability was demonstrated in this system. The maintenance of this state is essential to preserving the current rate of disease, and any intervention to attempt to reduce this rate must consider that such a state is vulnerable to perturbation. This may result in an unintentional rise in disease, either during the intervention, or more likely following any lapse in its application.

The maintenance of endemic stability in this region may be dependent on the maintenance of a constant relationship between two different species of *Theileria*, which are in turn, transmitted by two different tick species. Any perturbation in the relationship between these two tick species may lead to a state of endemic instability with an ensuing increase in ECF. This perturbation may be caused by a change in the use of acaricides, or the development of resistance in *A. variegatum*, but not in *R. appendiculatus*, or by a change in habitat that might affect *A. variegatum* and *R. appendiculatus* differently. The use of acaricides as a means of reducing disease in this region would not appear to be prudent.

The infect and treat method (ITM) for the prevention of ECF has been shown to substantially reduce losses due to ECF in pastoralist systems (Martins et al. 2010).

However, it has yet to be widely applied in Western Kenya, where more humid conditions lead to constant year round tick populations. It is worth considering the effect that ITM may have if widely implemented in Western Kenya. ITM is postulated to increase the carrier state of *T. parva*. As a tenet of the endemic stability paradigm, it may be that an increase in the carrier state may lead to an increase in the proportion of infected ticks, leading to increased infection pressure on calves. This may lead to an increase in clinical disease if calves are infected younger, and inoculated with a larger number of organisms from several attached ticks. If experimental evidence supports the relationship between *T. mutans* and *T. parva* then the use of ITM, or more importantly, a lapse in the use of ITM, may lead to an unexpected increase in clinical cases. This is because an increase in the carrier state of *T. parva* may affect the relative infection pressure of *T. parva* compared to *T. mutans*. Consequently, it may become more common for calves to become infected with *T. parva* before *T. mutans*. The tick stabilate used in ITM may need adjustment to local conditions, and may possibly be improved by the addition of *T. mutans*.

It should be concluded that an increased awareness of the impact of ECF in the region may be enough. If farmers were able to identify at risk calves early, and had a good knowledge of how best to treat those calves, there may be no need for population level intervention.

7.4 Conclusions

The aims of the IDEAL project were to improve understanding of the epidemiology of infectious diseases affecting cattle in tropical regions, to investigate how co-infections affected host outcome, and to investigate the concept that positive traits cluster within individuals. This thesis works to further the larger aims of the IDEAL project.

The main findings of this thesis were:

- Farmers had a poor understanding of the risks facing their calves.
- *T. parva* was a significant cause of clinical episodes and an important cause of mortality.

- Helminths were a significant cause of clinical episodes, and a substantial cause of mortality, and the host response was burden dependent.
- Clinical presentation of disease was complicated by the presence of co-infections.
- The hazard of infection was associated with elevation and herd size, which possibly influenced local variation in tick populations.
- Low birth weight was a risk factor for being a sick calf.
- Going out grazing, being older at infection, and possibly prior infection with *T. mutans* were all protective against death following *T. parva* infection.
- *T. parva* was endemically stable in the region.
- The maintenance of endemic stability may be influenced by the presence of *T. mutans*.

My priorities for onward work would be:

- Validation and development of diagnostic tools for both *T. parva* and *T. mutans*.
- Investigation of *T. parva* strains infecting calves over time, and possibly the MHC genotypes of the calves.
- Investigation of the local distribution of both *R. appendiculatus* and *A. variegatum*, and the infection rates of *T. parva* and *T. mutans* in those ticks.
- Further investigation of the carrier state, and the infection rate of ticks feeding from carrier animals.
- Experimental investigation of the effect of prior infection with *T. mutans* on clinical outcome following a subsequent *T. parva* infection.

Results presented in this thesis improve the understanding of the epidemiology of *T. parva* in the described population, and how it relates to other common infections in the region. *Haemonchus* and *T. mutans* were identified as possibly important co-infections with *T. parva* in terms of clinical expression and outcome. This work has identified a need for research into *T. mutans*, following the observation that the infection impacts

on growth (Thumbi 2012), and may be a cause of anaemia in young calves (Conradie Van Wyk, 2012), and may protect calves against mortality due to ECF (chapter 6). This work offers a platform from which to further investigate the paradigm for endemic stability of *T. parva*.

Appendices

Appendix A

Design and descriptive epidemiology of the Infectious Diseases of East African Livestock (IDEAL) project, a longitudinal calf cohort study in western Kenya

This paper was accepted to BMC Veterinary Research. Some of the figures from this paper are also in chapter 3.

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RESEARCH ARTICLE Open Access

Design and descriptive epidemiology of the Infectious Diseases of East African Livestock (IDEAL) project, a longitudinal calf cohort study in western Kenya

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Abstract

Background: There is a widely recognised lack of baseline epidemiological data on the dynamics and impacts of infectious cattle diseases in east Africa. The Infectious Diseases of East African Livestock (IDEAL) project is an epidemiological study of cattle health in western Kenya with the aim of providing baseline epidemiological data, investigating the impact of different infections on key responses such as growth, mortality and morbidity, the additive and/or multiplicative effects of co-infections, and the influence of management and genetic factors.
A longitudinal cohort study of newborn calves was conducted in western Kenya between 2007-2009. Calves were randomly selected from all those reported in a 2 stage clustered sampling strategy. Calves were recruited between 3 and 7 days old. A team of veterinarians and animal health assistants carried out 5-weekly, clinical and postmortem visits. Blood and tissue samples were collected in association with all visits and screened using a range of laboratory based diagnostic methods for over 100 different pathogens or infectious exposures.

Results: The study followed the 548 calves over the first 51 weeks of life or until death and when they were reported clinically ill. The cohort experienced a high all cause mortality rate of 16% with at least 13% of these due to infectious diseases. Only 307 (6%) of routine visits were classified as clinical episodes, with a further 216 reported by farmers. 54% of calves reached one year without a reported clinical episode. Mortality was mainly to east coast fever, haemonchosis, and heartwater. Over 50 pathogens were detected in this population with exposure to a further 6 viruses and bacteria.

Conclusion: The IDEAL study has demonstrated that it is possible to mount population based longitudinal animal studies. The results quantify for the first time in an animal population the high diversity of pathogens a population may have to deal with and the levels of co-infections with key pathogens such as *Theileria parva*. This study highlights the need to develop new systems based approaches to study pathogens in their natural settings to understand the impacts of co-infections on clinical outcomes and to develop new evidence based interventions that are relevant.

Keywords: Cattle, Infectious disease, Kenya, Longitudinal study, Cohort, Epidemiology, Study design

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Background

It is estimated that by 2050 the global population will have risen to 9 billion with much of this growth predicted to occur in sub-saharan Africa [1]. There is therefore an urgent need to improve food production in these regions, and livestock production constitutes an essential part of this. In addition to providing food through milk and meat, livestock also provide hides, draught power, manure for fertiliser, building and fuel, capital reserves and cultural services and in many marginal regions are the only useful way of utilising poor quality grazing land. Livestock are key to poor peoples' livelihoods and offer an important route out of poverty.

Constraints on livestock production are varied and include nutrition, management, access to markets, natural catastrophes and importantly infectious disease (eg. [2]). Sub-Saharan Africa (SSA) harbours 12 of the 15 former World Organisation for Animal Health (O.I.E.) list A diseases considered most contagious including African swine fever, Rift Valley fever and African horse sickness. In addition, many less contagious but arguably more important diseases such as East Coast fever, trypanosomosis, brucellosis and leptospirosis are widespread. A systematic literature review on causes of morbidity and mortality among smallholder dairy farms in Eastern and South Africa identified tick-borne diseases, diarrhoea and trypanosomiasis as the most commonly documented causes of mortality [3]. This limits production directly but also ensures these regions are unable to trade animals and their products in international markets [4]. However, rinderpest is a clear example where a regional approach has produced a highly successful eradication programme and the world is now rinderpest free [5]. This points to the need for targeted research to understand the full spectrum of disease problems in a farming system and how an integrated control package might release the genetic potential of the existing livestock while maintaining genetic resilience to environmental or emerging disease threats.

Previous work in infectious disease epidemiology has focused on single disease studies eg. Zhang [6], Bronsvoort [7] and Gachohi [8] or a few closely related diseases eg. [9] but, in reality, organisms are normally infected with a number of more or less pathogenic organisms at any one time. There is increasing scientific interest in how pathogens interact, within both individuals [10,11] and populations [12]. Examples include studies of viruses, bacteria, protozoa and helminth infections in both humans and livestock [11-20]. These interactions can be positive or negative and involve mechanisms such as: common risk factors and transmission routes (including shared vectors); non-specific immune responses; cross-reactive acquired immune responses; increased susceptibility of immuno-suppressed or immuno-compromised hosts; non-specific effects of genetic polymorphisms and nutritional deficiencies; the demographic and behavioural impacts of infectious diseases and of intervention measures. There may also be consequences of variations in the timing and ordering of exposure, infection, and disease caused by different pathogens, including responses to vaccinations [21,22].

Animal health research in SSA has traditionally focussed on specific infections, particularly tick-borne and tsetse-borne diseases, not necessarily because they are the major diseases of cattle kept by the poor in these environments, but because they are known historically to be serious constraints to commercial systems using improved breeds. However, livestock in the tropics are routinely exposed to a wide variety of pathogens [23] whose direct and indirect impacts on animal health are unlikely to be independent of one another. Local breeds have been reared in these heavy disease challenge settings for many centuries which has resulted in selection for broad disease resistance likely at the expense of higher production [24]. Yet there have been no integrated studies of the co-distribution, co-incidence and overall impact of the major infectious diseases of livestock in the tropics. There is a need for detailed knowledge of the burden of infectious diseases impose on livestock as a prerequisite to informed decision making, resource allocation, prioritisation of research and selection of interventions. However, there is growing evidence that disease impacts cannot be fully understood by reference to single infections in isolation [25]. Instead, a holistic approach is required which considers both direct and indirect interactions between pathogens and the effects of these on the epidemiologies of infectious diseases of cattle and of the disease burdens they impose and, ultimately, of their impacts on human welfare [16,26].

The Infectious Diseases of East African Livestock (IDEAL) project is a multi-disciplinary study which addresses two major issues: 1) the widely recognised lack of baseline epidemiological data on the dynamics and impacts of infectious diseases of cattle in the tropics; and 2) improving understanding of interactions between multiple infections and their sequelae by testing two specific hypotheses: i) that the negative impacts of different infections are not independent; ii) that 'positive' traits (e.g. resistance to infection, higher growth rates, low morbidity) cluster in certain individuals. In order to test these hypotheses we designed a longitudinal epidemiological field study to follow a random sample of newborn indigenous short horn zebu calves, with known genotype, through the first 12 months of life and to monitor them closely to identify when and what pathogens they were exposed to and the impact these had individually and in combination.

This paper describes the study design and reports the descriptive epidemiology of the IDEAL project. In particular we provide baseline data on the farm demographics and characterise the small holder African Shorthorn Zebu farming system of western Kenya which may be representative of the wider Lake Victoria basin. We also report the overall infectious disease related mortality rates and incidence of clinical episodes, the range of pathogens and exposures observed and the proportion of the cohort affected by each to provide a context for future papers on specific aspects of mortality and morbidity.

Methods

Study setting

There has been intensive work to define the distribution of different agricultural production systems in East Africa (eg. [27,28]). This study focused on a specific production system, sedentary mixed crop-livestock small holdings. This system encompasses >50% of poor people (defined as income below US$15 per month [29]) resident in East Africa [30], covers extensive areas of Kenya and beyond, and is of increasing importance as populations grow.

The study site was an area of western Kenya approximately 45 x 90km covering some or all of Busia (95.9%), Teso (96.3%), Siaya (55.5%), Butere/Mumias (26.9%) and Bungoma (20.4%) districts. Each district is further divided into sub-locations which are the smallest administrative unit in Kenya for which data was available on cattle numbers. A SL typically contains 60 to 90 households per km^2 and is 10-20km^2 in area. Land plots are typically 1-5 ha in size, with around 60% of households owning 2-3 breeding cattle grazed communally. The study site included 280 sub-locations (excluding 2 that were in Busia and Mumias towns) across 5 agro-ecological zones (AEZ). AEZ is a way to describe the type of land and its suitability for different crops and combines data on soil, topography, and climate. The areas of Kakamega, Vihiga, Lugari and Mt Elgon districts were not included as they were considered less representative of smallholder livestock farmers in East Africa (e.g. Mt Elgon slope, large-scale dairy farming more prominent) and due to logistic restrictions (i.e. the diagnostic laboratory was in Busia town, to which samples were transported daily).

Study design and recruitment

A stratified 2-stage random cluster sample of calves was drawn. The 1st stage cluster sample (by sub-location) was selected by random sampling sub-locations with replacement within each AEZ stratum. A total of 20 sub-locations were selected (Figure 1 and Table 1). A second stage sample size of 28 calves per sub-location was chosen at random to achieve the desired minimum sample size of 500 calves (based on logistical constraints and ability to detect a minimum relative risk of 3 with 80% power) and to allow for some losses (Table 2). A reporting system was established in each of the 20 selected sub-locations using a reporting pathway from Farmer → Sub-location-chief → Sub-chief → IDEAL Office. Each recruitment day the animal heath assistants (AHA) collated the eligible calf births for the sub-location and randomly selected 1-3 calves randomly from a hat each day. In order to be eligible the calf had to meet a set of specific selection criteria which were (1) the calf had to be between 3 and 7 days old at recruitment; (2) it was not as a result of artificial insemination; and (3) the dam was not managed under zero-grazing conditions. These criteria were set to give a reasonable window to capture calves being born without being too old and to avoid recruitment of exotic breeds rather than indigenous cattle. The sub-locations were visited on a rolling 5 week cycle to ensure there was an even distribution of calves across space and season. Calves were recruited over the 5 week cycle with 4/20 sub-locations being visited each week, taking 2 years to recruit the complete cohort. Only one calf per dam was recruited and a farmer could only have one calf at a time in the study. Recruitment was conditional on the farmer allowing access to the calf and willingness to report clinical episodes to the project and not "self treat". A flat rate of compensation was agreed with the local veterinary office for this. Owners were asked to call the IDEAL team if a calf was observed to be ill between visits and one of the project veterinary surgeons would examine the calf and treat if considered to be seriously ill or a welfare issue. Calves were censored after any visit where a treatment was begun.

Upon recruitment a household questionnaire was completed by interview with the owner/head of the household. The questionnaire included questions about the farm size, crops, water sources, and other livestock. The dam was examined and a form completed and if it or the calf failed any of the eligibility criteria, the calf was excluded. The calf was then examined and a recruitment form and routine visit form completed. The calf was examined for congenital deformities and excluded if any were found. This is summarised in Figure 2.

Data collection and training of data collectors

Data collection took place at the farm. A team comprising a veterinary surgeon/senior AHA and two AHAs went to each animal and followed a standard protocol for the physical examination and collection of compulsory samples. If the dam was also being visited there was an additional protocol for dam examinations. The AHAs were also trained in data collection and all questionnaires and data collection tools were piloted over

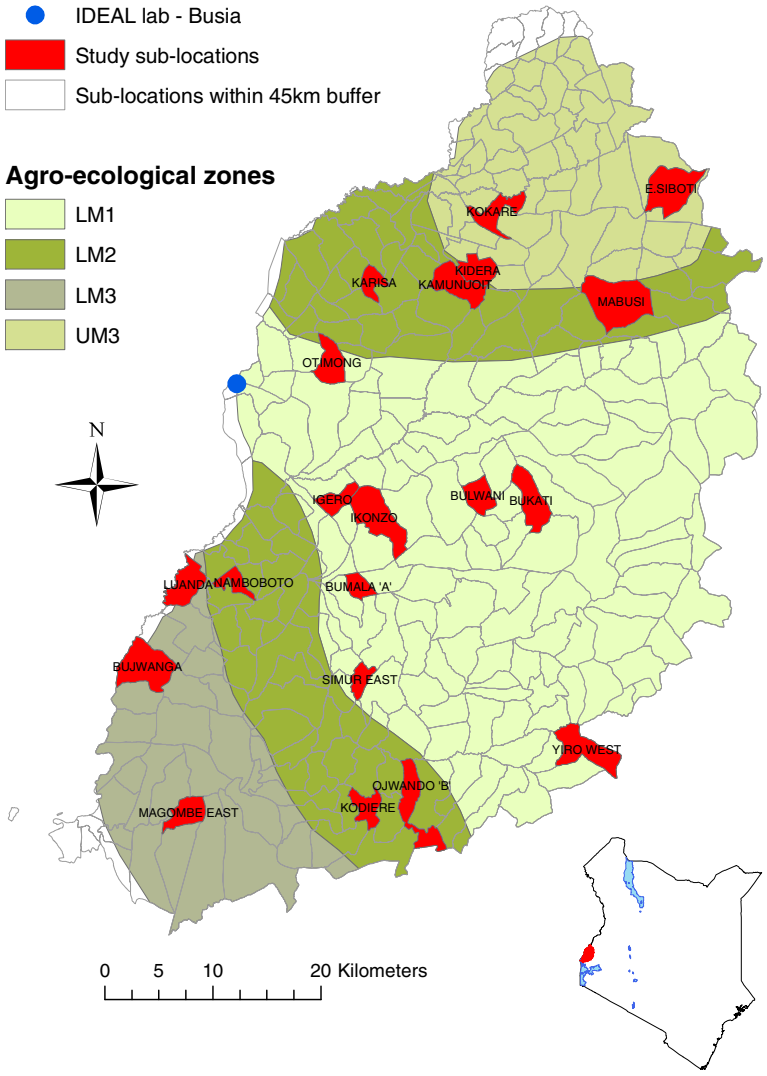

Figure 1 Map of western Kenya showing the study area, agro-ecological zones and sub-locations (selected sub-locations highlighted).

about 9 months during the set-up phase of the project in western Kenya. Data were collected via a hand held Palm OS® Personal-Digital Assistant (PDAs) and simultaneously on a paper questionnaire form. Barcodes were used to identify and link samples to individual animals. At the diagnostic field laboratory in Busia, data were downloaded from the hand held device to a database and cross-checked against the paper records and any discrepancies resolved with the AHA who collected the data.

Routine clinical examination of calves

The clinical examination consisted of a systematic physical examination of the calf. This included observation of the animal at rest, posture, alertness, rectal temperature, weight, girth, FAMACHA score [31], mucus membrane colour, skin elasticity, presence and species of ticks and other ectoparasites and full palpation of the body checking for lesions and discharges. In addition to the physical examination of the calf a short questionnaire was used to update other activities on the farm such as any animal purchases or sales, treatment of the other livestock or cases of illness in other livestock.

A standard set of samples were collected at recruitment (7D), 5 weekly (5W), and 51 weeks (Y) visits as detailed below. A marginal ear vein sample was used to make a thick and a thin blood smear to screen for haemoparasites and for manual differential cell counts following shipment to Pretoria University. A jugular vein sample was collected into plain tubes for total serum protein estimation using a refractometer (model RHC-200ATC, Westover Scientific) and storage for antibody screening for a range of

Table 1 Selected sub-locations with census/demographic characteristics (taken from the Human Population Census in Kenya 1999)

AEZ	Sub-location	No. households	Area(km^2)	Cattle density per km^2	Average herd size
UM3	East Siboti	1245	15.80	2439	3.4
	Kokare	325	8.29	937	6.1
	Kidera	314	7.36	728	4.8
LM1	Yiro West	1361	13.70	1187	3.9
	Simur East	415	4.32	425	3.8
	Igero	532	5.60	681	3.6
	Bumala A	724	4.38	222	2.3
	Ikonzo	1421	16.40	598	2.8
	Bulwani	478	6.87	578	3.2
	Bukati	993	11.20	1259	2.5
	Otimong	506	8.66	869	4.1
LM2 middle	Mabusi	1575	22.50	1575	3.1
	Kamunuoit	556	11.00	957	4.0
	Karisa	292	4.63	247	2.2
LM2 South	Ojwando B	832	12.60	1095	4.6
	Kodiere	630	6.38	849	4.7
	Namboboto	351	4.46	220	2.7
LM3	Luanda	726	9.76	730	4.7
	Bujwanga	1025	16.70	792	4.2
	Magombe East	578	7.67	852	5.4

haemoparasites, bacteria and viruses and 0.5ml was added to RNAlater® (Ambion®) and stored at 4°C. An EDTA sample with 'magic buffer' was collected for genomic analysis (7D only). An EDTA sample for: (a) DNA extraction for pathogens; (b) direct microscopy on thick and thin smears for haemoparasities and (d) routine haematology including WBC, RBC, PCV, MCV, HGB, MCH, MCHC using a Sysmex pocH-100iV Diff automated blood analyser (Sysmex® Europe GMBH) was also collected. A further EDTA sample was stored at -80°C until DNA extraction and shipping to Pretoria University for screening (Y or last visit before death) for a large range of blood borne parasites using the reverse line blot (RLB) [32]. A heparinised blood sample was collected for *Mycobacterium bovis* screening using the "Bovigam" ELISA (Prionics®, Celtic Diagnostics Ltd., Ireland) (Y only). In addition samples were collected for white blood cell stimulation, however, this was discontinued early in the study because of logistical constraints. Faecal samples were collected via rectal palpation for screening for helminths using standard techniques [33]. Samples were divided and one part put in a plastic bag and stored overnight at 4°C for screening by McMasters technique for strongyle eggs, by the direct Baermans technique for *Dictyocaulus vivperia* larvae, by Ziehl-Neelsen stained smear for *Cryptosporidium spp.* and *M. avium paratuberculosis* and by sedimentation for fluke species eggs. The second part was stored in a pot at room temperature overnight and then prepared for larval culture to speciate strongle eggs. Samples with >2000 coccidia oocyts were also cultured to type the species of coccidia present. Three superficial skin snips were taken from the ventral abdomen and incubated directly in RPMI-1640 (Sigma-Aldrich®) to screen for *Onchocerca spp.* microfilaria [34]. Results from diagnostic tests done in the field laboratory in Busia were entered directly in a separate laboratory database. In addition at the final visit to a calf a standard set of measurements of height at wither, nose to tail length and phenotypical measures such as coat colour and hump and dewlap were recorded. This is summarised in Figure 3.

Table 2 Distribution of sub-locations (SL) across agroecological zones (AEZs) in western Kenya and number selected for the IDEAL study

AEZ	No. SL/AEZ	Proportion/AEZ	No. SLs selected
LM1	114	0.40	8
LM2	86	0.30	6
LM3	28	0.10	3
LM4	4	0.01	0
UM3	53	0.19	3
Total	285		20

Clinical episodes and post mortem examinations in calves

In addition to routine clinical examinations and in order to capture as many clinical episodes as possible local AHAs working for the Kenyan Department of Veterinary Services in the sub-locations made weekly visits to each calf. These weekly visits involved a limited clinical examination focusing on identifying any acute disease and in particular any pyrexic or traumatic episodes. In the event that they identified pyrexia, enlarged lymph nodes or respiratory distress, they contacted an IDEAL project veterinary surgeon and an extra non-routine visit was made. The main triggers for a visit were a temperature of >40.5°C, generalised lymphadenopathy, anorexia, diarrhoea, generalised skin conditions, non-weight bearing lameness, coughing or respiratory distress. However each case report was considered and was visited depending on history and if there was believed to be a compromise in welfare. A full clinical examination was carried out and additional samples were collected based on the clinical syndrome observed. These included swabs of any discharges for bacteriological culture and

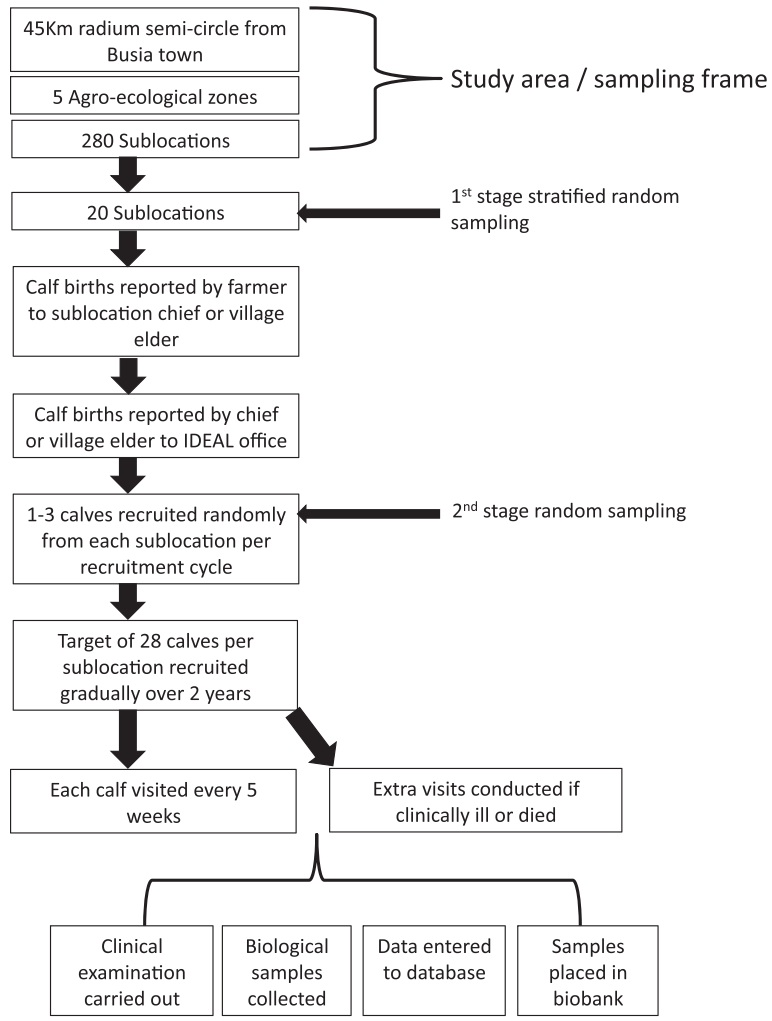

Figure 2 Schema showing the design and sampling used in the IDEAL project.

typing, viral swabs and heparin blood samples for virological culture, and needle aspirates from enlarged lymph nodes for microscopy. If calves were in a severely diseased state the project veterinarian used their professional judgement and a set of criteria agreed with the ethics committee at University of Edinburgh/International Livestock Research Institute and the animal was euthanised if necessary.

In the event that an animal died or was euthanised a full gross post mortem examination was carried out following standard veterinary approaches working through the body systems. A standard set of tissues was collected from each animal, including lung, liver, duodenum, ileum and lymph nodes, with additional samples specific to the suspected aetiology where appropriate. In the event of a history of sudden death a marginal ear vein blood smear was made and stained with methylene blue and checked for the presence of anthrax bacilli prior to further examination. In the event of a positive smear no post mortem was performed and the carcass buried. If there were neurological signs and/or a history consistent with rabies the head was removed and sent for testing at the Central Veterinary Laboratories at Kabete, Kenya and the remainder of the carcass incinerated. For those animals with neurological signs and no history of possible bites, a brain smear was prepared using the standard approach for identification of *E. ruminantium* the cause of heartwater disease.

Examination of the dams

In addition to the above the calf's dam was examined at each visit. At recruitment a full clinical examination was done (including manual palpation of the udder for evidence of mastitis), the girth measured and the animal was condition scored using a standard 10 point score [35]. In addition phenotypic measurements of coat colour

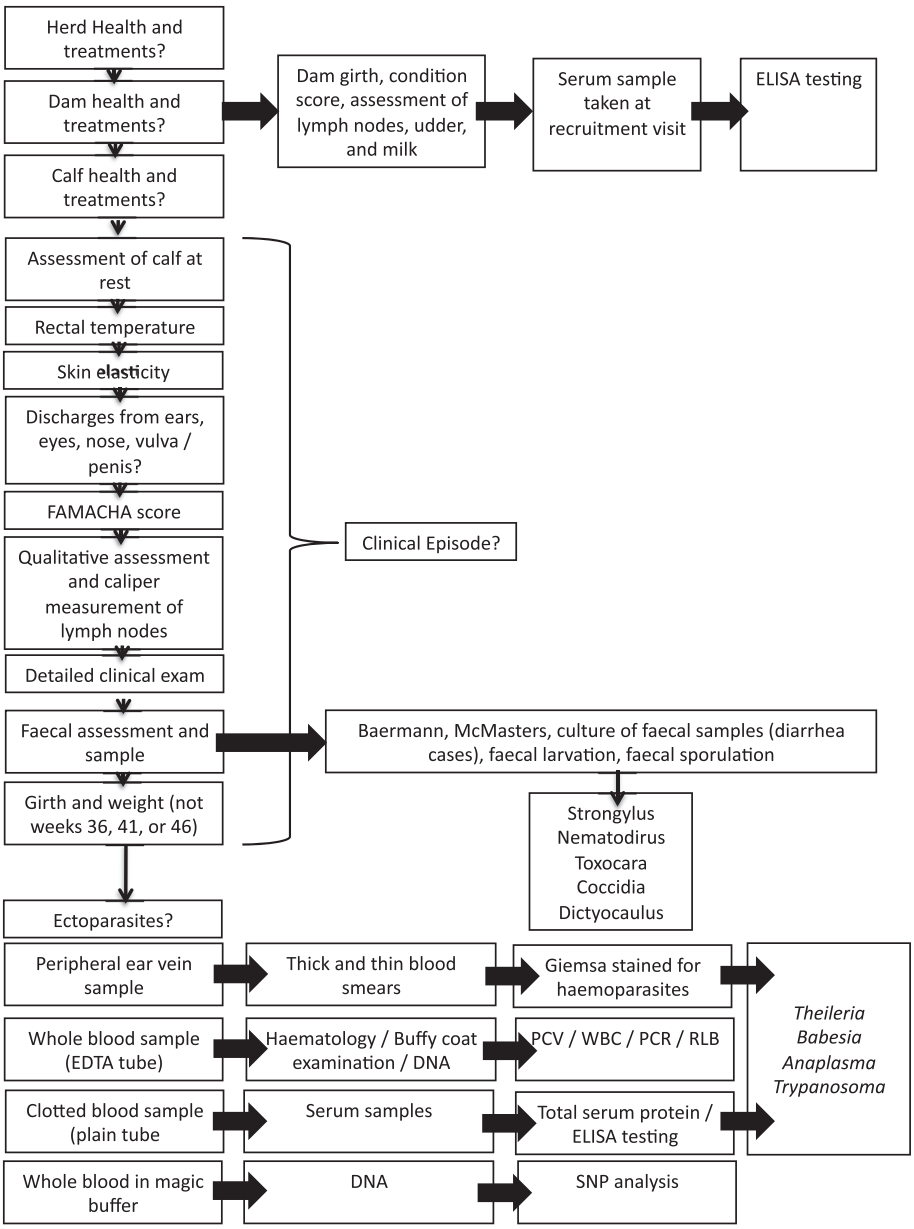

Figure 3 Schema showing the types and timings of clinical examination of calves and the types of sample collection for the IDEAL project.

and pattern, horn length and shape, ear shape, size of hump and dewlap were recorded. Two plain and 3 EDTA vacuutainers of blood were collected for possible use later. At each 5 weekly visit up to the visit after the calf was weaned the dam was re-examined, the girth was measured, the animal was condition scored and the udder examined.

In the initial phase of the study we attempted to collect milk samples from dams at each visit. These are low production animals and have very small udders and teats compared to a holstein for example. In the majority of cases we were unable to collect samples as the calf would have suckled before we arrived and/or the owner had milked the dam. Similarly the AHAs were initially trained to use the California milk test [36] but again it proved very difficult to get enough milk to test. Both these activities were suspended after the first 3 months in December 2007.

Laboratory analysis

A full list of pathogens that the project attempted to identify that we believed likely to be present in this setting is given in Table 3 and includes 100 different pathogens. The various techniques used and the time points at which they

Table 3 Pathogens screened for during the study

Pathogen	Test	Visits tested	Pathogen	Test	Visits tested
Actinomyces sp.	RB	CE	*Hepatozoon spp.* catch-all	RLB	Y
Actinomycetes	RB	CE	*Hyalomma spp.*	CL	7D, 5W, Y
Amblyomma variegatum	CL	7D, 5W, Y	*Hypoderma bovis*	CL	7D, 5W, Y
Anaplasma bovis	RLB	Y	*Klebsiella ozaenae*	RB	CE
Anaplasma centrale	RLB	Y	*Klebsiella pneumoniae*	RB	CE
Anaplasma marginale	RLB	Y	*Listeria spp.*	RB	CE
Anaplasma ovis	RLB	Y			
Anaplasma phagocytophilum	RLB	Y	Lumpy skin disease	PCR	CE
Arcanobacterium pyogenes	RB	CE	*Micrococcus spp.*	RB	CE
Babesia bicornis	RLB	Y	*Moniezia spp.*	FM,FC	7D, 5W, Y
Babesia bigemina	RLB	Y	*M. avium paratuberculosis*	ZN	Y
Babesia bovis	RLB	Y	*Nematodirus spp.*	FM,FC	7D, 5W, Y
Babesia caballi	RLB	Y	*Non-pathogenic Staphylococci*	RB	CE
Babesia canis	RLB	Y	*Oesophagostomum radratium*	FM,FC	7D, 5W, Y
Babesia divergens	RLB	Y	*Onchocerca spp.*	SNP,MIC	Y
Babesia felis	RLB	Y	*Ostertagia ostertagi*	FM+FC	7D, 5W, Y
Babesia gibsoni Japan	RLB	Y	*Pasteurella multocida*	RB	CE
Babesia microti	RLB	Y	*Rickettsia spp.* catch-all	RLB	Y
Babesia motasi	RLB	Y	*Rickettsia spp. (DnS14) raoultii*	RLB	Y
Babesia odocoilei	RLB	Y	*Riphicephalus appendiculatus*	CL	7D, 5W, Y
Babesia ovis	RLB	Y	Rotavirus	ELISA	CE
Babesia rossi	RLB	Y	*Salmonella spp.*	RB	CE
Babesia vogeli	RLB	Y	*Sarcocystis spp.*	HIS	PM
Bacillus anthracis	RB	PM	*Staphylococcus aureus*	RB	CE
Bluetongue virus	PCR	Y, CE	*Staphylococcus epidermicus*	RB	CE
Bacillus spp.	RB	CE	*Staphylococcus epidermidis*	RB	CE
Boophilus spp.	CL	7D, 5W, Y	*Staphylococcus spp.*	RB	CE
Borrelia afzelii	RLB	Y	*Streptococcus bovis*	RB	CE
Borrelia burgdorferi s. lato	RLB	Y	*Streptococcus spp.*	RB	CE
Borrelia burgdorferi s. stricto	RLB	Y	*Theileria annae*	RLB	Y
Borrelia garinii	RLB	Y	*Theileria annulata*	RLB	Y

Table 3 Pathogens screened for during the study *(Continued)*

Borrelia valaisiana	RLB	Y	*Theileria bicornis*	RLB	Y
Bunostomum trigonocephalum	FM	7D, 5W, Y	*Theileria buffeli*	RLB	Y
Bovine Viral Diarrrhoea Virus	ELISA - ag	Y	*Theileria cervi*	RLB	Y
Calicophoron spp.	FM,FC	7D, 5W, Y	*Theileria equi*	RLB	Y
Chabertia ovina	FM,FC	7D, 5W, Y	*Theileria equi*-like	RLB	Y
Clostridium spp.	RB	CE	*Theileria lestoquardi*	RLB	Y
Coccidia spp.	FM,FC	7D, 5W, Y	*Theileria mutans*	RLB	Y
Coccobacillary	RB	CE	*Theileria orientalis* 1	RLB	Y
Cooperia spp.	FM,FC	7D, 5W, Y	*Theileria parva*	RLB,PCR	Y
Corynebacterium spp.	RB	CE	*Theileria spp.* (buffalo)	RLB	Y
Cryptosporidium spp.	ZN,MIC	7D, 5W, Y	*Theileria spp.* (duiker)	RLB	Y
Dermatophilus congolensis	RB	CE	*Theileria spp.* (kudu)	RLB	Y
Dictyocaulus viviparus (L1)	FB	7D, 5W, Y	*Theileria spp.* (sable)	RLB	Y
E.coli	RB	CE	*Theileria spp.*	MIC, (RLB)	7D, 5W, Y, CE
Ehrlichia chaffeensis	RLB	Y	*Theileria taurotragi*	RLB	Y
Ehrlichia ruminantium	RLB,MIC,PCR	Y, CE	*Theileria velifera*	RLB	Y
Ehrlichia spp. (Omatjenne)	RLB	Y	*Toxocara vitulorum*	FM,FC	7D, 5W, Y
Eimeria alabamensis	FM,MIC	7D, 5W, Y	*Trichophyton spp.*	MIC	CE
Eimeria auburnensis	FM,MIC	7D, 5W, Y	*Trichostrongylus axei*	FM,FC	7D, 5W, Y
Eimeria bovis	FM,MIC	7D, 5W, Y	*Trichuris spp.*	FM,FC	7D, 5W, Y
Eimeria cylindrica	FM,MIC	7D, 5W, Y	*Trypanosoma brucei*	HCT,DG,PCR	7D, 5W, Y
Eimeria ellipsoidalis	FM,MIC	7D, 5W, Y	*Trypanosoma congolense*	HCT,DG,PCR	7D, 5W, Y
Eimeria subspherica	FM,MIC	7D, 5W, Y	*Trypanosoma spp.*	HCT,DG,PCR	7D, 5W, Y
Eimeria zuernii	FM,MIC	7D, 5W, Y	*Trypanosoma theileri*	HCT,DG,PCR	7D, 5W, Y
Epizootic haemorrhagic disease	PCR	Y, CE	*Trypanosoma vivax*	HCT,DG,PCR	7D, 5W, Y
Fasciola spp.	FS,MIC	7D, 5W, Y	*Weksella zoohelcum*	RB	CE
Haemonchus placei	FM,FC	7D, 5W, Y			

RB routine bacteriology, *CE* clinical episode, *CL* clinical examination, *RLB* reverse line blot, *7D* recruitment visit, *5W* routine 5 weekly visit, *Y* final visit at 51 weeks, *FM* faecal examination by McMaster's technique, *FC* faecal culture, *MIC* routine microscopy, *SNP* skin snip and culture, *ZN* Ziehl–Neelsen stain, *DG* dark ground microscopy, *HCT* haematocrit, *PCR* polymerase chain reaction.

were done are also provided for reference. In some cases there is overlap as some techniques will only differentiate to genus level while others will allow species specific identification.

In addition, the project screened stored sera from calves at 51 weeks or from their last visit prior to death for evidence of exposure to a number of other diseases believed likely to be important in this region. Further, plasma and DNA were analysed at a number of external laboratories (Table 4).

Whole blood samples in EDTA were stored in "magic buffer" (Biogen Diagnostica, Spain) and were genotyped using the Illumina 50K bovine SNP chip (Illumina Inc.®).

Table 4 Serological screening tests to pathogens

Pathogen	Ab/Ag based	Test name	Manufacturer	Visits tested
M. bovis	Ab	Bovigam ELISA	Prionics	Y
Respiratory Syncitial virus	Ab	ELISA	Svanova	Y
Bluetongue virus	Ab	ELISA	PI	Y
T. parva	Ab	ELISA	ILRI in house	7D, 5W, Y
T. mutans	Ab	ELISA	ILRI in house	7D, 5W, Y
A. marginale	Ab	ELISA	ILRI in house	7D, 5W, Y
B. bigemina	Ab	ELISA	ILRI in house	7D, 5W, Y
Parainfluenza 3 virus	Ab	ELISA	Svanova	Y
Bovine Viral Diarrhoea virus	Ab	ELISA	Svanova	Y
Bovine Viral Diarrhoea virus	Ag	ELISA	Svanova	Y
Epizootic Haemorrhagic disease virus	Ab	ELISA	PI in house	Y
Akabane disease virus	Ab	ELISA	PU in house	Y
Palyam group	Ab	ELISA	PU in house	Y
Infectious Bovine Rhinotracheitis virus	Ab	ELISA	Svanova	Y
Neospora caninum	Ab	ELISA	Svanova	Y
Brucella spp.	Ab	ELISA	IDEXX	Dam 7D
Leptospira hardjo	Ab	ELISA	Linnodee	Dam 7D

PI is the Pirbright Institute (formerly the Institute for Animal Health). *Ab* antibody, *Ag* antigen.

Database and sample tracking

The project managed data in a set of linked Access databases (Microsoft Corp.). All reports of calf births and recruitment visits were managed in the reporting database. After animals were recruited the main household questionnaire and the routine clinical visits, clinical episodes and post mortems were recorded using palm pilots running Satellite Forms (SatelliteForms.net). These were connected to the field database and daily downloaded. Every animal was tagged with a bar coded ear tag and visit sheets for each individual were kept. At every visit, the bar code was scanned to minimise recording errors. The field database generated a list of samples and then tests that were to be carried out on them in the local Busia laboratory and this was synchronised each evening so the laboratory staff knew what testing to do each day. The laboratory database linked all the barcoded samples in the field database to the respective calf, to the test results, to where the samples and any daughter samples generated from the original field sample were stored and when they were moved to the ILRI lab in Nairobi or to other laboratories outside Kenya. At the end of the field work the field and laboratory databases were merged and moved to a multiuser MySQL database that could be accessed and updated remotely giving all staff access to the data for analysis. All samples eventually were moved to ILRI Nairobi and were appended to the ILRI laboratory information management system for sample management and tracking. Samples where possible were stored in duplicate and only one of the duplicates moved at a time to reduce the risk of losing complete sample sets. At ILRI duplicates are stored in separate buildings in either -20°C or -80°freezers or in vapour phase in large liquid nitrogen biobank chambers as appropriate.

Tropical Livestock Units

Tropical Livestock Unit (TLU) is a standardising measure used to quantify different types and sizes of livestock. It gives a reference unit that captures the total number of livestock units present in a farm, with 1 TLU being the equivalent to an animal of 250 kg liveweight. One TLU is equivalent to 1 cow, 10 goats or sheep, 5 pigs, 100 chickens, and 0.7 camels [37,38]. This unit has been used for different purposes, including calculating insurable livestock units in the index-based livestock insurance programmes in northern arid areas of Kenya. The different species and sizes of livestock kept in the farms were converted in to TLU's to serve as a proxy indicator for livestock wealth of each household. The conversion factors used here are those reported by Njuki *et al.* [39].

Analysis

The R software version 2.9.1 (http://cran.r-project.org/) was used to generate the descriptive statistics and graphics

of the farm characteristics and frequencies of pathogens. All statistical tests were interpreted at the 5% level of significance.

Survival time for each calf was defined as the age at which the study calf died due to infectious causes. Animals that died for reasons other than infectious causes, or that were lost or removed from the study before one year for non-compliance were censored. These contributed "at-risk" time only up to the censoring point. All survivors to one year were censored at the time of leaving the study. Kaplan-Meier estimates of the survival function were used to determine the overall mortality rates [40].

Results

Cohort characteristics

A total of 548 calves were recruited and followed for up to 51 weeks or until they died over the 3 year period of the field work. The spatial distribution of the selected sub-locations is given in Figure 1 and the number of calves recruited as a proportion of the breeding dams in each sub-location is given in Table 2. The cattle densities in each sub-location ranged from 220/km^2 to 2439/km^2 and the sub-locations ranged in size from 4.38 km^2 to 22.5 km^2. The average herd size across all sub-locations ranged from 2.2 breeding cows in Karisa a more hilly area compared to 6.2 animals in Kokare. The life line for each calf is illustrated in Figure 4 and highlights the drop out of calves from death and euthanasia and the pattern of clinical episodes. In addition there were 2 periods where sampling and particularly recruitment were suspended. The first was following the political unrest in 2008 and work in the field was suspended for 6 weeks. This resulted in a small number of calves missing visits for one or two 5 weekly visits. The second was over an extended holiday period in 2009/2010.

Farm characteristics

A total of 548 owners/household heads were interviewed. Data on the owner's age, gender, education and training level attained, and main occupation are summarised

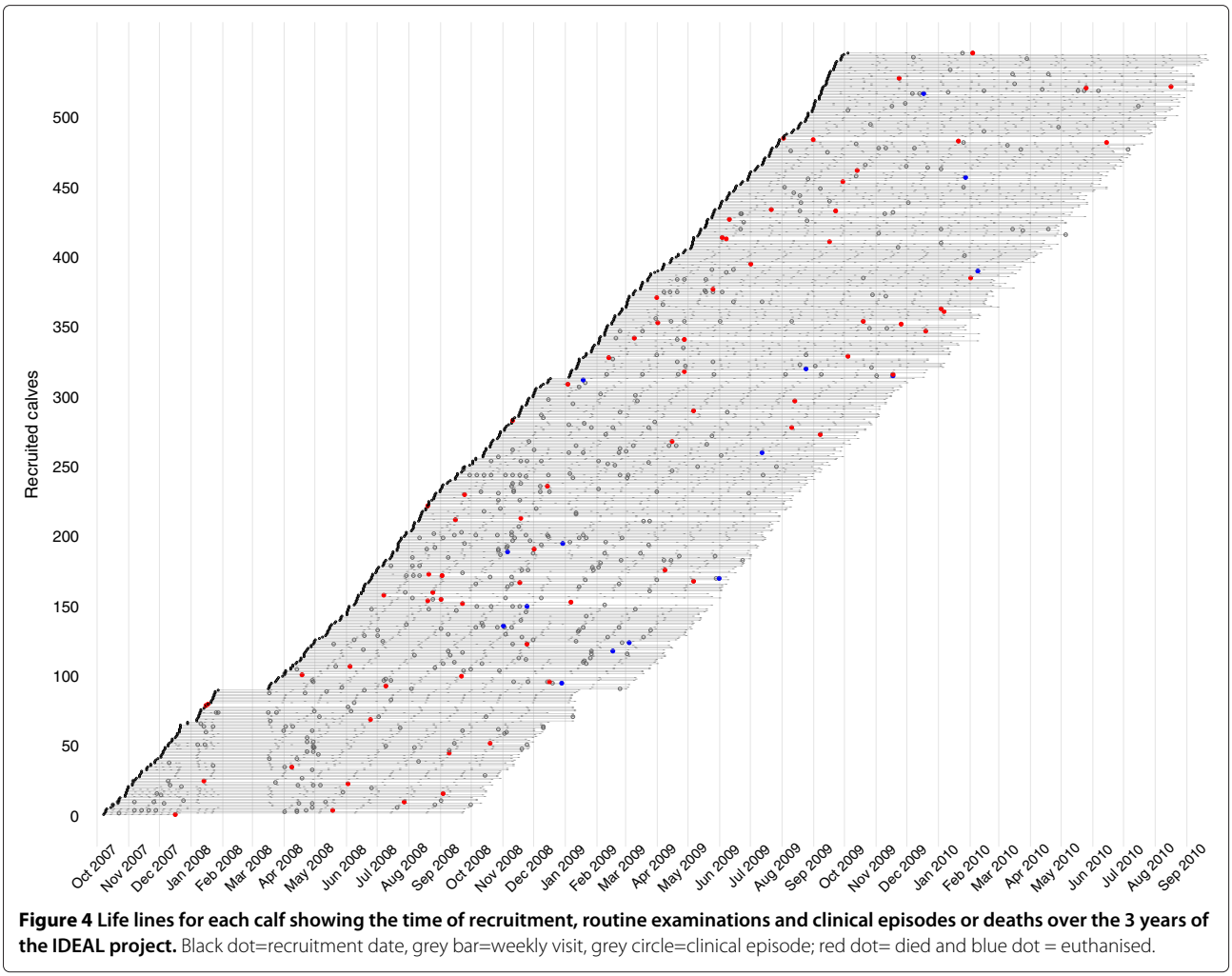

Figure 4 Life lines for each calf showing the time of recruitment, routine examinations and clinical episodes or deaths over the 3 years of the IDEAL project. Black dot=recruitment date, grey bar=weekly visit, grey circle=clinical episode; red dot= died and blue dot = euthanised.

in Figure 5 and table 5. Of the 548 owners, 69% were men and 31% women. The mean age in years for male owners was 50.7 (range 22 - 85) and that for females 49.0 (range 20 - 78). Differences in ages between male and female farmers were statistically insignificant ($p = 0.1679$, df= 352, 2-sample t-test). Approximately 15% of the farmers had no formal education, and none had attained university education. A small percentage (21%) had gained technical skills allowing them to work in the informal markets with the common ones being masonry, tailoring and carpentry. The majority (86.2%) of the interviewed owners reported farming as their only source of income, with the rest reporting teaching, civil service, pension and business as their main sources of income with farming offering supplementary income.

The average farm size was only 1.98 ± (0.1 SE) hectares (range 0.1 to 23.1 ha), with majority (96.1%) being owned. Such land is continuously sub-divided, to give adult sons an inheritance and ownership rights. This practice results in families owning small pieces of land that are sometimes not economically viable for agriculture. The rest (3.9%) rented the land they farmed on. All the farms selected for the study kept cattle and also planted food crops, with each farm having a median 5 (range 1 to 131) cattle. The indigenous short horn zebu cattle were the predominant breed kept, with only a small percentage (3.1%) also keeping zebu crosses. Farmers kept more than one species of livestock; an attribute identified as a strategy for spreading risk of losses [41,42]. Different livestock species serve different purposes within the farm enterprise. The general herd structure is given in Table 6, with adult females comprising 41.4% of all cattle kept, and adult males 9.8%.

Husbandry and management practices

Almost 60% of the farms provided housing for livestock. This was usually in the form of an open yard/kraal

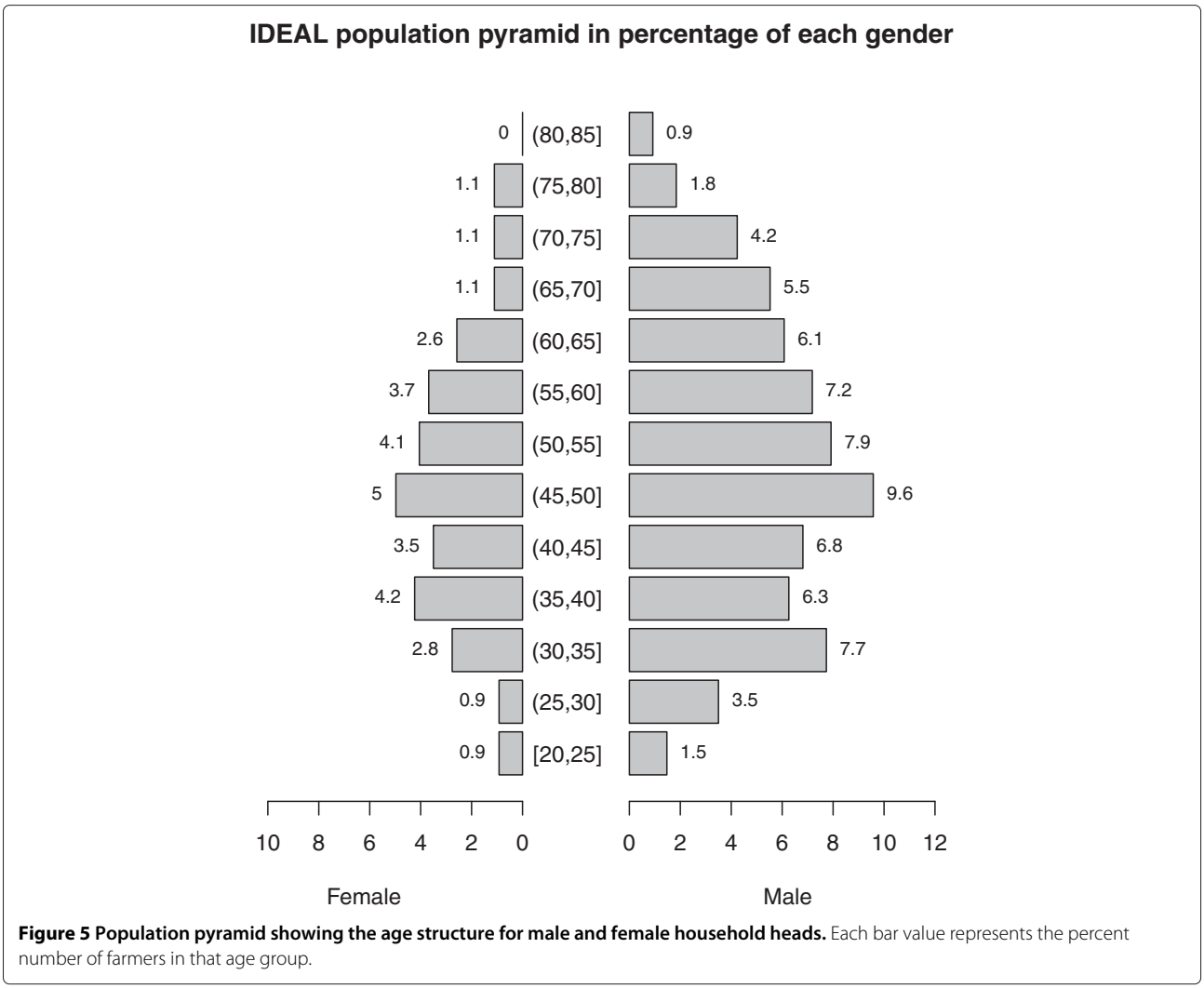

Figure 5 Population pyramid showing the age structure for male and female household heads. Each bar value represents the percent number of farmers in that age group.

Table 5 Descriptive statistics for farmer's demographic variables

	N*	Frequency	Percent**
Sex of house head	548		
Male		370	69
Female		178	31
Education level of house head	544		
None		81	14.9
Primary education		337	61.9
Secondary education		126	23.2
University education		0	0
Technical training	541		
No		415	76.7
Yes		126	23.3
Main occupation	544		
Farmer		469	86.2
Teacher		6	1.1
Civil servant		11	2
Business		22	4.1
Retired with pension		14	2.6
Other		22	4

*Not all the farmers responded to the questions in the questionnaires and N notes the number of respondents to the particular question.
**The proportions are calculated using the number of respondents to the question.

surrounded by a fence made of untreated wood or bushes with no roof. The remaining 40% of farms provided no housing and the animals were left free or tethered within the homestead during the night. Among those providing housing, 83.1% housed calves separate from the dams/bulls. Calves were not allowed to graze with adults (in 94.4% of the farms) until after weaning. This was mainly to prevent calves suckling dams while out in the field. Calves were allowed to suckle as the farmer milked, with some farmers reporting that milk-let-down in their short horn zebus only happened when stimulated by calves. Other farmers obtained their share first and left the rest for the calf to suckle.

During the dry season, 49.1% of the farms reported providing drinking water for the cattle within the homestead. The rest drove their animals to a water source. These proportions did not differ significantly between the dry and the wet seasons. Distances to the watering points were below 1 km for 73.8% and 75.8% of the farms in the dry and wet seasons respectively, with the rest travelling more than 1 km to access drinking water. Table 7 shows data on the housing, distances to watering points, frequency of watering, and quality of water both in the dry and wet seasons.

Table 6 Land sizes, livestock species kept and the herd structure

	N	Percent	Mean	median	s.d.	Min.	Max.
Land size owned (hectares)							
	517	94.3	1.98	1.37	2.28	0.1	23.1
Livestock numbers							
All cattle	548	100	6.5	5	7.6	1	131
Indigenous cattle	548	100	6.5	5	7.6	1	131
Cross breds	17	3.1	1.4	1	1	1	5
Goats	209	38.1	3.5	3	3.8	1	33
Sheep	112	20.4	3.9	2.5	5.3	1	48
Pigs	150	27.3	2.2	1	2.2	1	13
Chickens	485	88.5	14.3	10	12.7	1	120
Dogs	297	54.2	2.04	2	1.4	1	9
Tropical livestock units	546	99.6	5.8	4.1	6.71	0.48	114.3
Herd structure (indigenous)	548	Frequency	Mean/farm	Percent			
Adult females		1463	2.7	41.4			
Adult males		345	0.6	9.8			
Female calves		465	0.8	13.2			
Male calves		446	0.8	12.6			
Weaning females		399	0.7	11.3			
Weaning males		417	0.8	11.8			
Total		3535	6.5	100.0			

Table 7 Description of housing, and watering practices in the dry and wet seasons

	N	Dry season		Wet season	
Housing	545	Freq	Percent	Freq	Percent
Kraal/yard		321	58.9	322.0	59.3
None		224	41.1	223.0	40.7
Access to water	547				
Distance to furthest watering point					
At homestead		91	16.6	100.0	18.3
<1 km		313	57.2	314.0	57.5
1–5 km		141	25.8	131.0	24
6–10 km		2	0.4	1.0	0.2
Frequency of watering					
Freely available		11	2	13.0	2.4
Once a day		149	27.2	446.0	81.5
Twice a day		367	67.1	87.0	15.9
Thrice a day		20	3.7	1.0	0.2
Water quality					
Good, clear		533	97.4	508.0	92.9
Muddy		14	2.6	39.0	7.1

Table 8 Location of trading markets and sources of breeding bulls

	N	Freq	Percent
Location of purchasing point	504		
Within sublocation		75	14.9
Neighbouring sublocation		396	78.6
Other		33	6.5
Purchasing point	539		
Market		533	98.9
Neighbouring farm		6	1.1
Breeding practices	548		
Own bull (bred)		63	11.5
Own bull (bought)		45	8.2
Bull donated		2	0.4
Bull borrowed		422	77
Communal area bull		19	3.5
Other		1	0.1

Cattle trading and breeding practices

Almost all the cattle purchases and sales (98.9%) were done through cattle markets (Table 8). The rest (1.1%) of the farms reported trading animals directly with neighbouring farms. A total of 24 different cattle markets were reported serving the 20 sub-locations, spanning four administrative districts. However, a quarter (6/24) of these markets served 71.2% of all the farmers in the study, an indication that farmers preferred trading in big markets, where they are likely to get more competitive prices.

There were no reports of organised breeding programmes, and farmers did not keep any written breeding records. The choice of breeding bulls was mostly based on availability of a bull, and if more than one then the farmer decided on personal preferences. Only 11.4% and 8.2% of the farms kept own-bred or purchased breeding bulls respectively (see table 8). Most farmers (76.2%) borrowed breeding bulls whenever their cows needed service. Based on this, only a few bulls are available to serve animals, raising the chances of widespread inbreeding. A few farmers (3.4%) indicated they did not make any direct breeding decisions and depended on their cows being served while grazing in the same communal areas or at watering points. This number is likely to be much higher than reported as animals mix freely and frequently at watering points and communal grazing fields.

Access to veterinary services

During the farmer interview at the recruitment visit, most farmers (84.7%) reported accessing some form of veterinary services, mainly provided by private animal health workers, and to a lesser extent by government animal health workers, and veterinary drug suppliers (see Table 9). A few farmers indicated they did not use the services of an animal health worker, and instead treated their sick animals themselves. Approximately 90% of farmers reported using some form of tick control of which most (89.9%) reported using whole body spraying with acaricides at the farm. Only a few farmers reported accessing communal cattle dips. Most of the cattle dips in the study sub-locations are abandoned and not in use. Only just over 50% of farmers reported using any form of anthelminthic treatment and only around 18% reported using any form of tsetse control. A moderate proportion of farmers reported using vaccination (52%) although most of those reporting using vaccines did not know what vaccine they had given their animals or what they were protected against (76.7%) and their use seems to be largely driven by need rather than a regular programme of control.

There was a notable difference between the proportion of farmers who reported carrying out disease control measures such as tick control and worming during the initial visit, and the actual proportion of farmers who reported using any preventive measures during the one year follow up period. This suggests that farmers are answering what they think they should be doing or maybe have done but a significant proportion actually then appeared to not carry out these measures over the course of our

Table 9 Description of access to veterinary services and disease control practices in the farm as reported during the calf recruitment visit

		Frequency	Percent
Access to veterinary services	544		
Yes		461	84.7
No		83	15.3
Type of Veterinary support	461		
Private animal health worker		264	57.3
Government animal health worker		176	38.2
Veterinary drug supplier		23	5.0
Farmer		10	2.2
Tick-control	548		
Yes		498	90.9
No		50	9.1
Application method	498		
Spraying whole body		462	92.7
Spraying legs only		9	1.8
Pour on		6	1.2
Hand dressing		25	5.0
Dipping		8	1.6
Other (traditional,manual removal)		10	2
Worm control	548		
Yes		309	56.4
No		239	43.6
Application method	309		
Drench		265	85.8
Bollet		47	15.2
Others (injectables/unknown)		2	0.6
Traditional		5	1.6
Trypanosome control	548		
Yes		98	17.9
No		450	82.1
Method used	98		
Spraying whole body		51	52.0
Chemotherapy		32	32.7
Pour-on		10	10.2
Other (dipping/head dressing/ unknown)		8	8.2
Use of Vaccines	546		
Yes		284	52.0
No		262	48.0
Frequency of use	277		
Routinely		9	2.9
When need arises		269	97.1
Vaccine type used	284		
Unknown		230	81.0
Anthrax		8	2.8
Black quarter		11	3.9
Contagious Bovine Pleural Pneumonia		1	0.4
Foot and mouth disease		25	8.8
Lumpy skin disease		18	6.3
Other		6	2.1

observations. Interestingly with tsetse control stated and observed activities seem to align well possibly reflecting the recent inputs form NGOs in this area. In contrast vaccination use was much higher than stated and this is not clear why such a discrepancy should arise. This highlights the need for caution in interpreting responses especially from cross-sectional data (see Table 10).

Morbidity and mortality

The 548 recruited calves contributed a total of 175,732 calf days of life to the study. Figure 4 shows the temporal pattern of deaths and clinical episodes over the 3 years of the study. A total of 88 calves died before reaching 51 weeks of age giving an crude mortality rate of 16.4 (13.2-19.5) per 100 calves in their first year of life (Table 11).

Fifteen calves were euthanised and were considered to have died from the primary pathology reported on *post mortem*. The distribution of times of deaths by

Table 10 Table comparing the proportion of farms reporting using each disease control measure at initial visit alongside actual proportion of farms that carried out the measures during the follow up period (n = 548)

Type of control	Initial visit %	Actual practice %
Tick control*		
Yes	90.9	69.9
No	9.1	30.1
Worm control*		
Yes	56.4	26.8
No	43.6	73.2
Tsetse and trypanosome control		
Yes	17.9	14.1
No	82.1	85.9
Vaccine use*		
Yes	52.0	96.4
No	48.0	3.6

*significantly different at the 5% level using a McNemar's chi-squared test.

Table 11 Counts of primary cause of deaths attributed by expert committee

Cause of death	No. calves
East coast fever	32
Unknown	20
Haemonchosis	9
Heartwater	6
Trauma	3
Actiomyces pyogenes	1
Babesiosis	1
Bacterialpneumonia	1
Black Quarter	1
Cassava	1
Foreign body	1
Mis-mothering	1
Rabies	1
Salmonellosis	1
Trypanosomiasis	1
Turning sickness	1
Viral pneumonia	1
No post mortem carried out	6
Total	88

NB 2 additional calves were considered to have died with ECF as a secondary contributing cause; one with heartwater and another with black quarter.

AEZ is given in the Kaplan-Meier plot (Figure 6) showing that AEZ5 which is UM3 in Figure 1 and includes Magombe East, followed by AEZ1 (LM1) which include Bumala A had much higher death rates than other AEZs. The reasons are not yet clear and are the subject of ongoing analyses. Deaths were also attributed to a secondary or contributing cause of death when this was appropriate.

Unfortunately due to logistical reasons *post mortems* were not carried out on 6 of these calves so their cause of death remained unknown. Of the remaining 82 deaths all received a post-mortem examination. A further 4 of this 82 were of unknown cause (a total of 10 calves that died of a completely unknown cause). Seven died from a known non-infectious cause (cassava poisoning (1), foreign body pneumonia (1), mismothering (1), starvation (1), trauma (3)) and 1 died from an unidentified non-infectious cause. Eleven calves had clinical signs indicative of an infectious agent but the definitive cause remained unidentified and 59 died of an infectious cause that was diagnosed by post-mortem examination, appropriate testing and clinical history (East Coast fever (33), turning sickness (1), haemonchosis (10), heartwater (6), babesiosis (1), rabies (1), salmonellosis (1), trypanosomiasis (1), black quarter (1), viral pneumonia (1), multifocal abcessation due to *Actinomyces pyogenes* (1), and *Arcanobacterium* infection (1). This gives a minimum of 70 deaths attributable to infectious diseases and a minimum mortality rate due to infectious causes of 13.3% (10.4-16.2) per 100 calves in the first year of life.

Of the 32 cases of East Coast Fever 8 had a contributing cause of helminthiasis, 5 of which were due to haemonchosis and 2 of trypanosomiasis. Of the 10 cases of haemonchosis 2 had a contributing cause of Theileriosis, and 1 of lung worm (*Dictyocaulus viviparous*). Of the 6 heartwater cases 1 had a contributing cause of East Coast fever as did the case of black quarter. It is interesting to note that in an area generally considered to have high tsetse challenge there seemed to be little clinical trypanosomiasis.

A further 307 clinical episodes were observed by the AHAs on their routine 5 weekly visits and 216 clinical episodes were reported during non routine visits in response to reported illness. The details of all the clinical signs and patterns is currently under analysis but the overall distribution of clinical episodes by age is given in Figure 7. This suggests a bimodal pattern with a large peak around 16 weeks at the time when maternal antibodies might be expected to be waning. There is a second smaller peak later around 41 weeks when many calves are weaned.

Pathogens and exposures

Figure 8 shows the list of pathogen/test combinations experienced by the calf by the time of publication crudely stratified into endoparasites, haemoparasites, bacteria and viruses. Some of the common pathogens such as *Theileria spp.* appear several times as a number of techniques were used to identify them. In addition, some assays, such as microscopy, do not distinguish between species. More detailed analysis of these co-infections is on going. What this Figure shows very clearly is that this population of calves is infected with over 50 different pathogens and has been exposed to at least a further 6 bacteria and viruses. However, relatively few pathogens were found in the majority of calves, and the main pathogens were helminths and protozoan haemoparasites. What is of particular interest is that, given such high incidences of these key pathogens such as *T. parva*, *A. marginale*, *B. bigemina* and *H. placei*, why more of these calves did not die. One of the main objectives of the continuing analyses of this dataset is to unravel the coinfections and relate these to the calf genotype and key outcomes such as growth rate, morbidity and mortality. It is also interesting that there are very few bacterial diagnoses and these appear to have only sporadic occurrence and rarely contributed to death. We plan to look in more detail at the dam serology, but of the 2 bacterial pathogen exposures already measured in the dams, *Brucella spp.* and

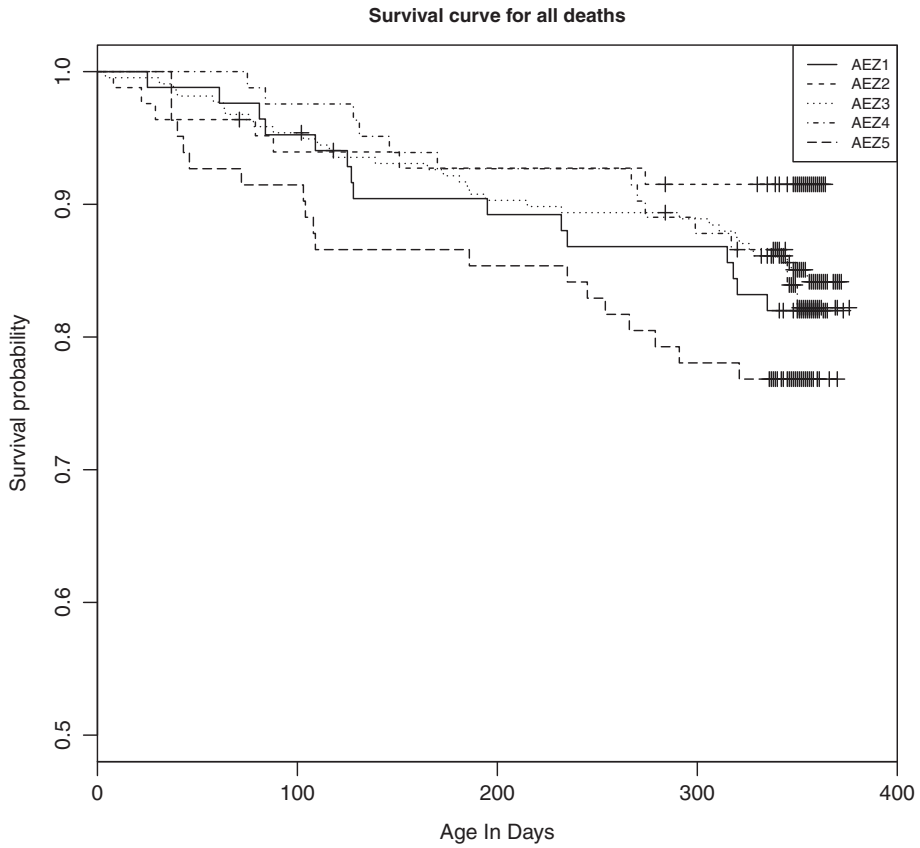

Figure 6 Kaplan-Meier survival curves for deaths due to all causes. + mark censoring for reasons other than death and are mainly at 51 weeks when visits stopped. The dashed lines give the 95% confidence intervals on the survival probability.

Leptospira hardjo the seroprevalences were extremely low, 0.036 (0.022-0.050 adjusted 95% CI) and 0.068 (0.035-0.101 95% adjusted CI) respectively. Also there was little clinical evidence of some of the major viral diseases such as foot-and-mouth disease.

Discussion

The IDEAL project is the first attempt to describe the entire disease burden of any naturally occurring population. Funding was only available to follow calves for the first 12 months of life. The use of a longitudinal design, though enormously logistically challenging in this environment, allowed us to generate a unique dataset to study the effects of co-infections in the SHZ breed in this small holder setting. This may be applicable across a large sector of the Great Lakes basin where very similar breeds and husbandry are in operation.

When designing the project a number of different approaches were considered. They included stratification by management system, wealth/herd size, livestock distribution, location, ethnicity, etc. However, the lack of available data on several of these factors led to the decision to stratify by agro-ecological zone only. Random cluster sampling will have ensured that reasonable representation was provided for the various levels of each of the un-stratified factors, i.e. the total sample size will include farmers with varying herd sizes and management systems. The proportion of sub-locations sampled in each AEZ is in proportion of each AEZ in the total survey area (based on numbers of sub-locations). The study was constrained by logistics to an area of 45 km radius from Busia town in order to make repeated visits possible. Initially other options were reviewed but following piloting of sampling in the field it became clear that given the road conditions and number of animals that would have to be sampled per day at the peak of sampling in year 2 this was the most practical approach.

Owners were paid a retainer for the year to allow access to the animals and therefore compliance was very high. There were a small number of instances of animals being stolen and of owners treating the calves with anthelminthics without consulting the project vet. Where these were identified animals were censored and their data from the visit following treatment discarded.

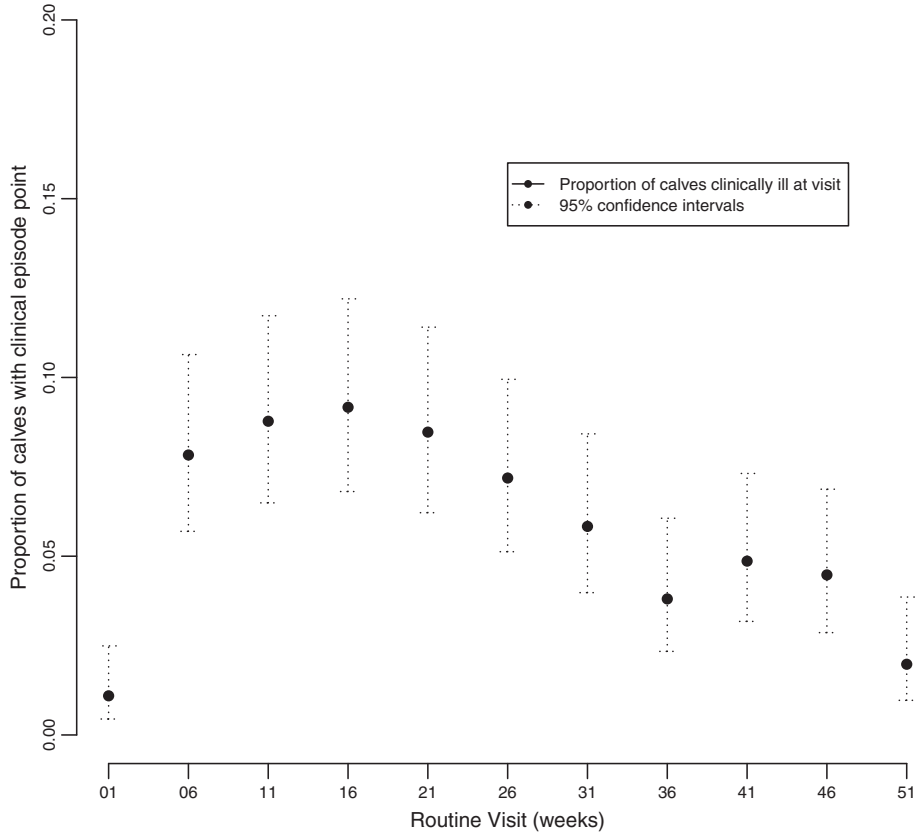

Figure 7 The distribution of the proportion of calves classed as having a clinical episode stratified by visit number over the 51 weeks of observations for each calf in the IDEAL project.

The descriptive analysis from the recruitment interview indicate that livestock production in this system is characterised by low-input, with as few as 30% of the farms carrying out any form of disease control during the follow-up time. Even for those farms that reported carrying out disease control measures, the frequency of these per year was below what would be effective. This level of management would likely be insufficient to support the use of improved "exotic" breeds which are kept in the region but which we intentionally excluded from this study. The Western Province of Kenya accounts for only 4% of Kenya's total exotic dairy herd [43]. This is despite major breed improvements programs instituted to support smallholder farmers in the region through increased livestock productivity [43,44].

Livestock disease and vector control are required for increased livestock productivity, and prevention of losses through disease-related morbidity, mortality, and loss of markets for livestock products. The observed lack of disease control has implications on some of the strategies envisaged to rapidly improve livestock-dependent livelihoods. It also highlights the need to provide support not just for the imported exotic breeds but also for the indigenous breeds in order to minimise the losses and maximise productivity. The consistent use of disease control practices has contributed to the relative success of the smallholder dairy sector in the Kenyan highlands [45]. The benefits of such controls, carried out at community level, have also been demonstrated in other settings [46]. Failure to consider these disease issues is recognised as a factor that could seriously reduce rural growth [47].

Many countries in sub-Saharan Africa have had to make structural adjustments to their veterinary infrastructure and the services they provide which leaves farmers and herdsmen without the support needed to introduce exotic genetic stock. Further, Rege *et al.* [48] argue that breeding strategies in the context of smallholder farms should be based on improving food security, income and overall livelihoods of the livestock keepers and should not be based on genetic improvement of livestock. Focus should be on providing the most appropriate genotypes in a local context. However, identifying these appropriate genotypes is itself complex. Mwachara *et al.* [49] identify the need to involve the livestock keepers in designing the breeding programmes to take into account the full array of contributions to livelihoods that these animals

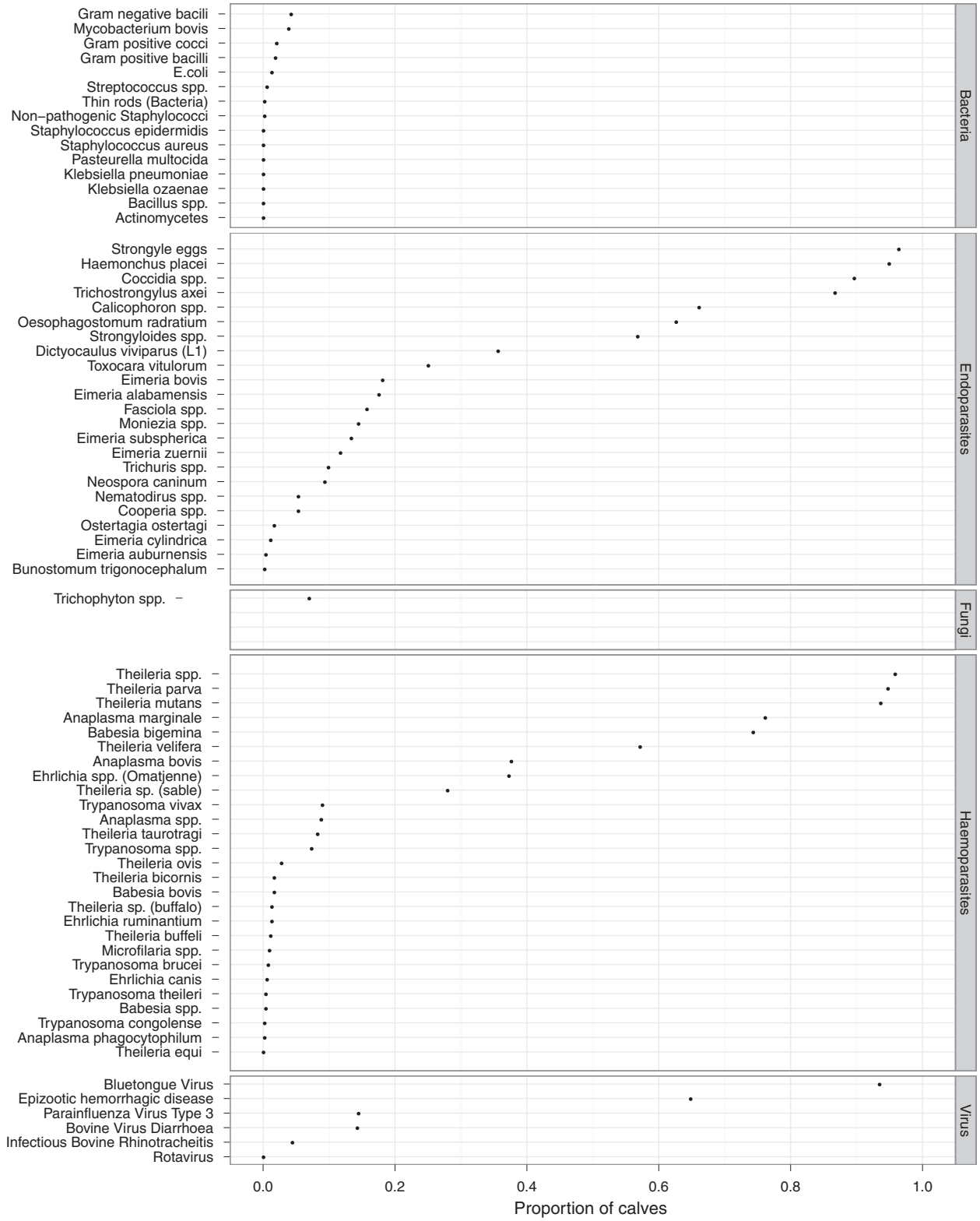

Figure 8 The proportion of animals positive for a given pathogen/test combination at any time through the course of the 51 weeks of observation on each calf in the IDEAL project.

make and so identify genetic characteristics related to these functions. Whereas most programs have concentrated on cross-breeding, there exists a lot of potential and advantages for improvements based on within-breed selection.

The mortality rates in this indigenous calf population were higher than anticipated at the design stage. There are few reports that we could find from similar systems but other reports from the region suggest a range of mortalities. Barnett [50] reported a mortality rate of 29% from a study based in Western Province Kenya. In a Tanzanian smallholder dairy system mortality rates of 35% were reported [51] within the first year with 42% reported as of unknown cause and 19% due to redwater (babesiosis). Swai *et al.* [52] reported mortality rates of 12% in small holder dairy systems in Zimbabwe with 56% ascribed to tick borne disease particularly east coast fever. Gitau *et al.* [53] reported 7% mortality in calves up to 6 months of age from the same area of Western. A more recent large study of calf mortality in Mali [54] reported an overall calf mortality of 17% but when this was broken down by system the more intensive systems had high mortality rates of 19% and 25% compared to 10% in the traditional pastoralist systems. Interestingly they report gastrointestinal disorders as causing 28% of their overall mortality followed by perinatal problems (16%) and accidents (14%). Direct comparisons are very difficult to make with many of these studies as the design, breeds, environment etc are not the same. However, it is useful to get an overall impression of how these animals are performing in this system. The mortality rate in the IDEAL cohort appears high given it is an indigenous breed that might be expected to have had time to adapt to the conditions. There are likely to be many contributing causes including possible inexperience in raising cattle compared to traditional cattle owning groups such as the Maasai or Fulani and the co-infection combinations present in the region.

The identification of pathogens at all time points in the study is on going. We adopted a very pragmatic approach using the best field techniques available as the method of diagnosis but for many pathogens this is not sufficient. For example speciation of *Theileria* parasites requires more detailed analysis such as RLB [55]. It must be noted that detection of pathogens is limited by the sensitivity of the assay, the presence of the pathogen at the time of sampling and its location in the tissue which is sampled. This presents many challenges in trying to produce a definitive list of pathogens at every time point for each calf. For this preliminary presentation of the pathogens we have simply summed across all visits to estimate the proportion of calves with each pathogen (or pathogen/test combination). This ignores the dynamics of the order of exposure but this is to be reported in a number of other papers. The list of pathogens is extensive but there are actually only a few very high prevalence pathogens. Theses are mainly gut helminths and tick borne haemoparasites, in particular *T. parva*.

The IDEAL project provides unique data on total livestock disease burden in the region, which will allow for ranking of infectious diseases in order of importance. Such data are important for prioritising interventions, the absence of which up to this point has led to a lack of metrics to assess the impact of livestock diseases leads and therefore inefficient resource allocation [2]. In addition, the project will provide data on the within breed variation of key traits such as growth rates, clinical tolerance and resistance, and survival. This provides a basis for identifying desirable traits that may be taken up while designing within-breed improvement programs. Within-breed selection may not achieve increased productivity per animal as rapidly as cross-breeding methods, but offers the opportunity to retain the adaptive characteristics already present in indigenous breeds and which may offer opportunity for adaptation to changing climates. The findings of positive associations between knowledge of diseases and access to veterinary support with whether farmers carry out disease control practices supports the idea that increased extension services would have significant positive effect on livestock productivity.

Conclusions

This population of calves is the first to have a comprehensive investigation of the pathogen burden and exposures of any animal population. The analyses of the biobanked samples will continue and it is expected that there will be further pathogens added to the list. This preliminary report on the IDEAL project design and data collection offers an overview of the farming system in Western, Kenya, and of the infectious disease challenges experienced by the calves of the region. It provides a detailed description of the methods used to collect this detailed longitudinal dataset. This provides more information for those reading analytical papers from the project, and acts as a supporting document to the extensive biobank held at ILRI, Nairobi. It gives preliminary results and offers an overview of more detailed analyses that result from the IDEAL project.

Competing interests

We declare that non of the authors at the time of the study or preparation of the paper have any competing interests that could influence or bias the content of this paper.

Authors' contributions

MW conceived the original idea and design and directed the project; MB was responsible for the study design, development of the clinical tools such as questionnaires, sample flows and diseases to be screened, data management and analysis and training of field staff; JP was responsible for the design and random sampling protocols, database management and data analysis; IH was responsible for management of the database, data extraction and analysis; HK was responsible for parasitology protocols and overall managing the project,

the sample flows and diseases to be screened for and biobank management; PT was responsible for the management and interpretation of the serological screening of samples; KC was responsible for the clinical protocols, postmortem protocols, interpretation of clinical and postmortem data and training of the field staff; and OH and MN were responsible for managing the phenyotypic protocols and analysis and interpretation of the genetic data components. ST, IC and AJ were the veterinary surgeons in the field and as well as carrying out the clinical work and managing staff on the ground participated in the development of the tools and interpretation of the results. OT designed and implemented the database for the project and contributed to the design of the study. All authors read and approved the final manuscript.

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Appendix B

A live weight - heart girth relationship for accurate dosing of east African shorthorn zebu cattle

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ORIGINAL RESEARCH

A live weight–heart girth relationship for accurate dosing of east African shorthorn zebu cattle

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Abstract The accurate estimation of livestock weights is important for many aspects of livestock management including nutrition, production and appropriate dosing of pharmaceuticals. Subtherapeutic dosing has been shown to accelerate pathogen resistance which can have subsequent widespread impacts. There are a number of published models for the prediction of live weight from morphometric measurements of cattle, but many of these models use measurements difficult to gather and include complicated age, size and gender stratification. In this paper, we use data from the Infectious Diseases of East Africa calf cohort study and additional data collected at local markets in western Kenya to develop a simple model based on heart girth circumference to predict live weight of east African shorthorn zebu (SHZ) cattle. SHZ cattle are widespread throughout eastern and southern Africa and are economically important multipurpose animals. We demonstrate model accuracy by splitting the data into training and validation subsets and comparing fitted and predicted values. The final model is weight$^{0.262} = 0.95 + 0.022 \times$ girth which has an R^2 value of 0.98 and 95 % prediction intervals that fall within the ±20 % body weight error band regarded as acceptable when dosing livestock. This model provides a highly reliable and accurate method for predicting weights of SHZ cattle using a single heart girth measurement which can be easily obtained with a tape measure in the field setting.

Keywords East African shorthorn zebu · SHZ · Weight estimation · Cattle · Heart girth · Dosing · Kenya

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Introduction

The accurate estimation of livestock weights is important for many purposes such as determining ration amounts, agreeing on sale prices and for ensuring the correct therapeutic dosing of animals. East African shorthorn zebu (SHZ) are multipurpose animals that serve as sources of draught power, milk and meat and contribute to household incomes throughout eastern and southern Africa (Rege et al. 2001). Milk production is largely determined by reproductive performance, which is in turn closely correlated with cow weight

and body condition (Kanuya et al. 2006). Similarly, the relationship between live weight and capacity for work in SHZ used as draught animals is well established (Bartholomew et al. 1994; Fall et al. 1997). It can thus be deduced that body weight can be used to evaluate the value of an animal intended for use as breeding stock, milk production, draught power or beef. A simple, accurate method of approximating SHZ body weight in the field will thus give farmers greater bargaining authority at cattle markets, maximising the economic return on the investments made in their animals.

Livestock pathogens, such as *Trypanosoma* sp., *Babesia* sp., *Anaplasma* sp. and *Theiliera* sp., remain important constraints to livestock production in east Africa, where the main control methods available to farmers are pharmaceuticals (Perry et al. 2002; Malak et al. 2012). However, underdosing of therapeutic pharmaceuticals not only fails to control pathogens but also leads to the development of antimicrobial-resistant bacteria through selection pressure (Spellberg et al. 2008; Morgan et al. 2011). Although the risk to human health has not been clearly defined, it is a major public health concern that the resulting antimicrobial-resistant genes will be transferred to bacteria pathogenic to humans, such as *Campylobacter* spp., *Salmonella* spp. and enterotoxigenic *Escherichia coli*, and enter the food chain or environment (Shuford and Patel 2005; Mathew et al. 2007; Gousia et al. 2011; Oliver et al. 2011). This may be of even greater concern in developing countries, where antimicrobial treatment options are limited by cost and availability (Okeke et al. 2005). Though rarely reported in the literature, there is also a risk associated with overdosing, which could lead to insufficient drug withdrawal times and increased risk of meat and milk residues, in addition to being wasteful and economically inefficient. Many of these issues are cause for concern in developing countries where access to reliable estimates of weight for dosing can be difficult to obtain and where the impacts of resistance are likely to be most severely felt and least likely to be monitored or controlled.

Weighing scales, though accurate, are not commonly available nor convenient for use in an African field setting. There are many studies (Buvanendran et al. 1980; Nicholson and Sayers 1987; Nesamvuni et al. 2000; Goe et al. 2001; Abdelhadi and Babiker 2009; Ozkaya and Bozkurt 2009; Yan et al. 2009) that have aimed to estimate weights from various body measurements, but these often require several measurements per animal, which is inconvenient, time-consuming and possibly dangerous (many animals in these settings are not as familiar with handling as European counterparts). European-based weigh tapes developed for Holstein or other European beef breeds consistently overestimate the true weight of SHZ cattle, which have very different conformations (Mwacharo et al. 2006; Machila et al. 2008). It is clear that the morphologically distinct SHZ, which comprise the majority of cattle in eastern and south-central Africa, will need their own predictive model of weight, and these may need to be complex functions over the full age range. Further, it has been found (Machila et al. 2008) that farmers consistently underestimate the live bodyweight of cattle, demonstrating the need for the development of an accurate and inexpensive method. Visual estimation of live weight in many livestock species is generally regarded as very inaccurate and prone to error. This manuscript uses statistical methods to develop and validate an accurate, statistical model for SHZ cattle live weight based on heart girth measurement.

Materials and methods

Data for the model came from two sources: a convenience sample of 241 cattle was selected at a number of livestock markets (Amukura, Kemodo, Funyula, Myanga, Ogalo, Bumala, Lugulu, Boro, Kocholya and Myanga) during June and July 2010, where some attempt was made to exclude animals with exotic genes through a seller questionnaire asking about origins and breeding, and at Mkura market in September 2010. All markets were in the Busia administrative district in a region of western Kenya near the Kenya–Uganda border. A further 462 observations were taken from the Infectious Diseases of East Africa (IDEAL) calf cohort study. Calves from 20 randomly selected sub-locations within a 45-km radius of Busia town were recruited and followed for the first 12 months of life. Each animal was observed at five weekly intervals, but to avoid issues of repeated measures, a single observation per calf was randomly drawn. The final data set therefore consisted of 703 SHZ cattle owned by smallholder farmers in western Kenya ranging in age from 1 week to fully mature. Each animal was weighed using either a pair of portable calibrated weigh beams (ZEMIC, model H8C-C3-1.5t-4B-SC) or a spring balance (for IDEAL calves up to 31 weeks of age). Their heart girth was measured using a simple measuring tape held with 1-kg tension using a light spring balance. Each animal's sex and age (estimated via dentition in the case of market animals) were also recorded.

Statistical analysis was carried out using the R software program (R Development Core Team 2011). The

Table 1 Model equations of form weight = given equation, breed applied to and source

Breed	Equation	Source
Fulani	$1{,}513 - 37.97x + 0.3093x^2 + 0.000749x^3$	Buvanendran et al. (1980)
Gudali	$-438 + 4.88x - 0.001823x^2$	Buvanendran et al. (1980)
Boran	$-432.73 + 4.81x$	Nicholson and Sayers (1987)
Nguni	$16.58 + 0.81x$	Nesamvuni et al. (2000)
Abyssinian SHZ	$-363 + 4.17x$	Goe et al. (2001)
Baggara	$-92.472 + 2.4573x^a$	Abdelhadi and Babiker (2009)
Holstein	$-473 + 5.21x$	Ozkaya and Bozkurt (2009)
Brown Swiss	$1{,}733.22 - 19.84x + 0.07x^2$	Ozkaya and Bozkurt (2009)
Crossbred	$-935 + 7.69x$	Ozkaya and Bozkurt (2009)
Holstein–Fresian	$-666.6 + 6.373x$	Yan et al. (2009)
SHZ	$-409 + 4.55x$	Kashoma et al. (2011)

Notation has been standardised so that x always refers to cattle heart girth
[a]The model in question used heart girth around the hump

data set was divided into two subsets, chosen by random selection, a modelling subset of slightly less than 50 % of the data was used to develop the statistical model and a validation set was used to validate the model. A likelihood maximised Box–Cox transformation, $h(y, \lambda) = (y^\lambda - 1)/\lambda, \lambda \neq 0$, was used to estimate the transformation power coefficient (Box and Cox 1964). Linear regression models were then applied to the transformed data to test the significance of potential explanatory covariates. A number of models obtained from the literature were also fitted (Table 1) and compared with the model developed in this study. The validation data set was used to determine the performance of the model predictions.

Results

The complete data weight versus heart girth scatter plot is provided in Fig. 1, showing the modelling and validation data subsets. The modelling subset of 300 observations was used to fit the transformation and regression parameters. The Box–Cox transformation parameter, λ, was estimated to be 0.262. Gender, weaning status, interactions and higher order girth terms were found to be not significant once heart girth was in the model. A very simple linear model was fitted to the transformed data. The model is given as

$$y_i^{0.262} = 0.95 + 0.022x_i, \quad (1)$$

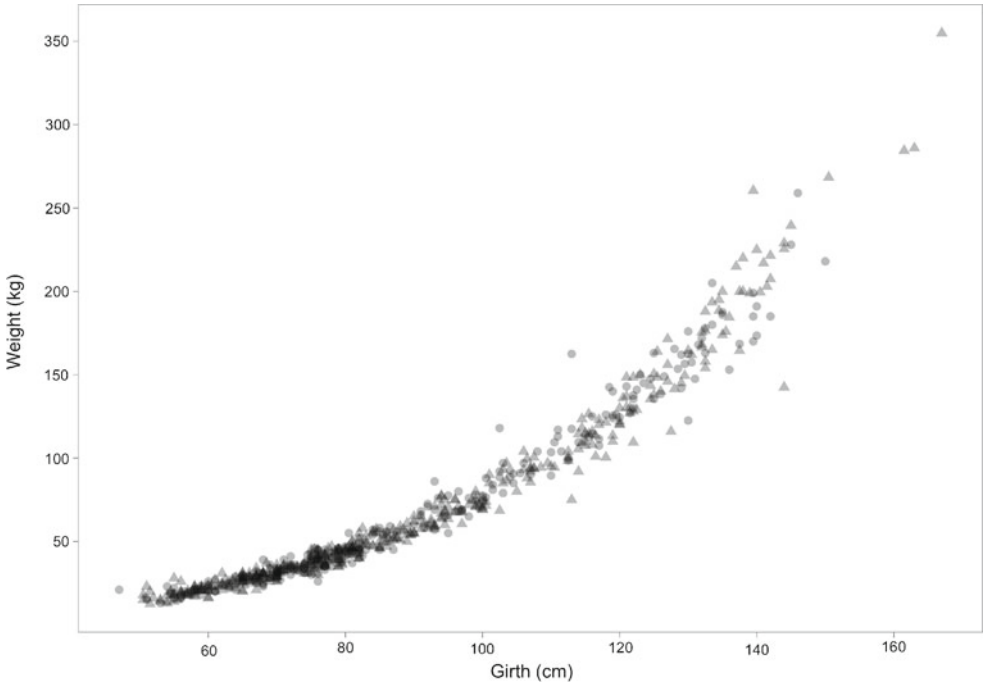

Fig. 1 Measured heart girth versus measured weight scatter plot for 703 African shorthorn zebu cattle. The model data set is indicated by *circles* and the validation data by *triangles*

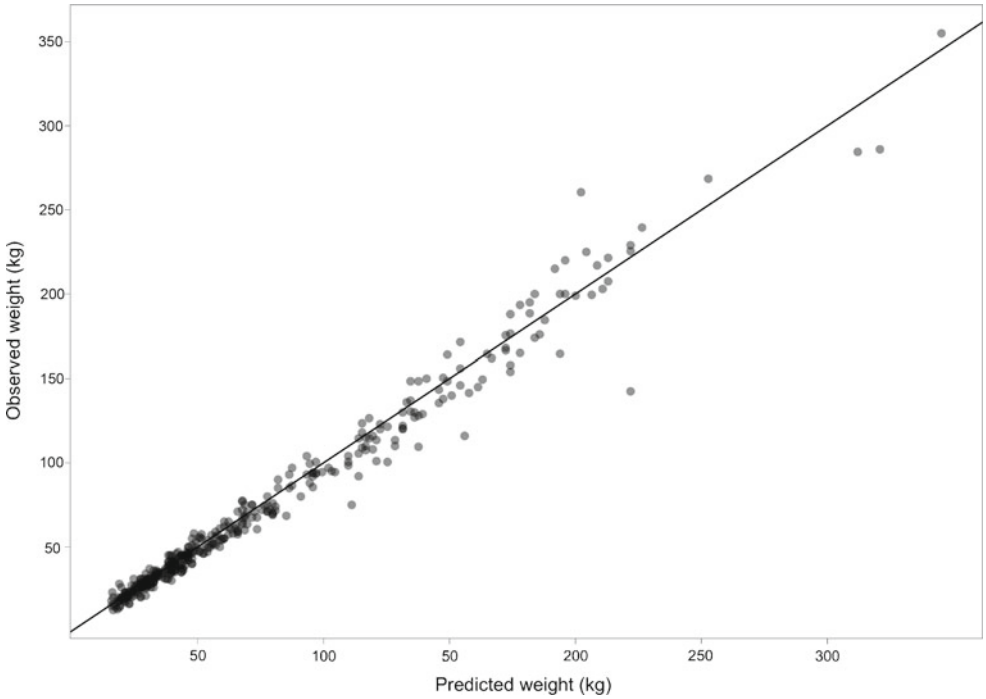

Fig. 2 Agreement between predicted weights applying the model developed on the training data set to the validation data set and the observed weights. The line of perfect agreement is overlaid on the plot

where x_i is the measured heart girth (in centimetre) for subject i and y_i is the measured weight (in kilogram) for subject i. This resulting model had an adjusted R^2 of 0.98 and a residual standard error of 0.08.

The model was then used to predict weights based on heart girth measurements in the validation data set. Figure 2 shows the agreement between predicted weights and observed weights in the validation data set. 95 % prediction intervals were calculated and compared with the 20 % safe dosing zone, as established by (Machila et al. 2008), and this is shown in Fig. 3 where the model, prediction intervals and safe dosing zone have been back transformed for clarity. Figure 4 shows the result of fitting models from the literature to

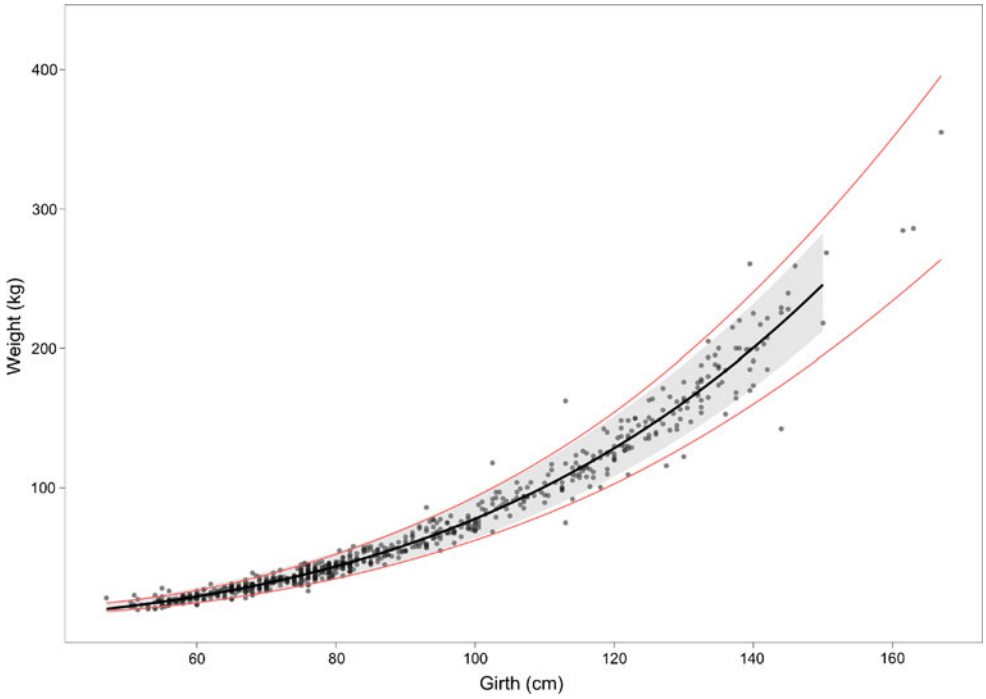

Fig. 3 Complete data overlaid with the best fit model (*thick solid line*), 95 % prediction intervals (*grey band*) and the ±20 % body weight safe zone for dosing (*thin solid lines*). The model line and 95 % prediction intervals are those developed from the modelling data subset only

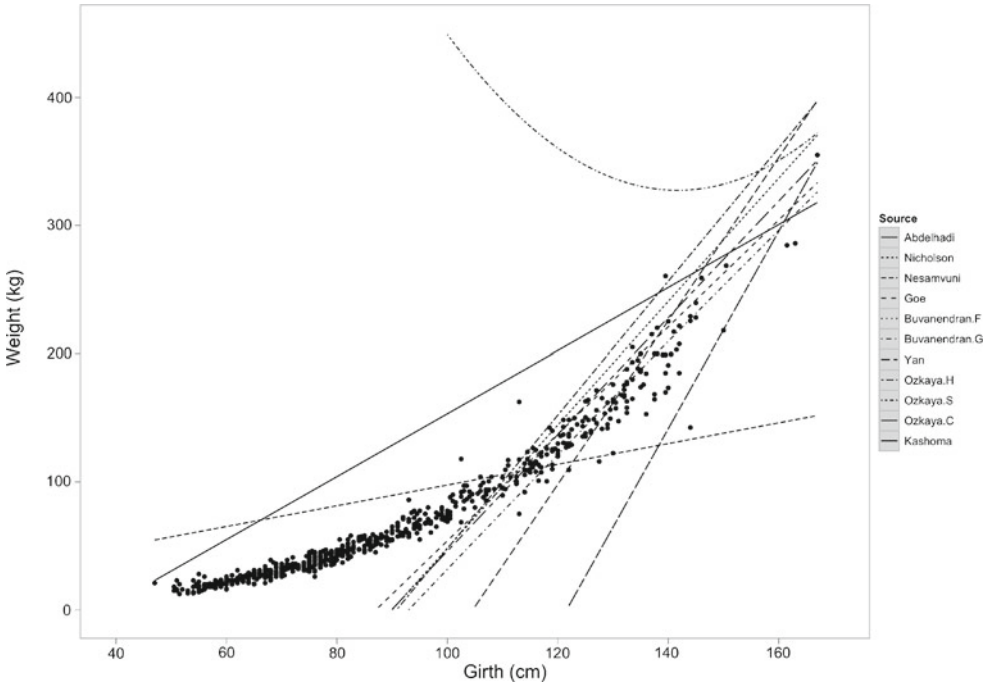

Fig. 4 Complete data overlaid with models sourced from the literature. Model equations can be found in Table 1

the complete data set; the model equations, breeds and citations can be found in Table 1.

Discussion

The proposed model for estimation of weight via heart girth measurements satisfies statistical and practical considerations. The model is highly significant, has a very high adjusted R^2 value, shows very good performance on the validation data set and, importantly, predicts weights for which the 95 % prediction intervals fall within the safe dosing zone. Because the model does not use sex or age to stratify results, it is very amenable to transfer onto a weight tape that can be used for all ages of SHZ cattle. The potential impact of this tool for smallholder farmers throughout eastern and southern Africa is far reaching, including the accurate dosing of animals to prevent selection for antimicrobial resistance, as well as accurate estimates of slaughter weight at market, which is the primary determinant of market price (Scarpa et al. 2003). As the data presented here show a clear nonlinear relationship between heart girth and weight and the majority of the literature-sourced models are linear, it is not surprising that they were not accurate at estimating weight for this data set. It is possible that breed conformation and animal maturity also play a role in the failure of other weight estimation models to fit the data as our animals. This analysis highlights the need to have appropriate measurement tools for different breeds and the dangers of trying to extrapolate from a model into a different breed and over a different age or size range; however, it seems clear that nonlinear models must be considered. Further research is required to determine if our model can be applied to other subgroups of zebu throughout Africa.

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Appendix C

Main Household Questionnaire

This questionnaire was carried out at the recruitment visit and gave details about the household into which the calf was born, the calf owner (farmer), the crops and land ownership, the herd and other stock kept on the farm, and the husbandry practices of the farm.

1. Initial Details

Calf ID _____ **AHA / Vet ID** _____

Dam ID _____ **Farmer ID** _____

Picture of calf at Recruitment: ☐

1.1 Date: _____ **1.2** Time: _____

1.3 Language of Questionnaire Administration: _____

Language code: 1: Teso; 2: Samia; 3: Bukusu; 4: Luo; 5: Swahili; 6: English; 7: Others [Specify] ; ND: ND

RPT Number: _____

2. Farmer Details

2.1 First Name: _____ **2.2** Surname/s: _____

2.3 Sex: ☐ Male ☐ Female **2.4** Age [years]: _____ ☐ ND **2.5** DOB: ___/___/___ ☐ ND

3. House Hold Details

3.1 Sub-location Name: _____ **3.2** Village Name: _____

3.3 GPS Coordinates [DD]:

Easting: _____ Elevation: _____

Northing: _____

4. Calf Details

4.1 DOB: ___/___/___ ☐ ND **4.2** Time of Birth: ___:___ ☐ ND **4.3** Sex: ☐M ☐F ☐ND

5. Dam Details

5.1 Age as estimated by farmer [Years] : _____ ☐ ND **5.2** Number of calvings [Including this calving] : _____ ☐ ND

5.3 Time of dam in farm [Months] : _____ ☐ ND **5.4** Was the dam milked on the week before calving? ☐ Yes ☐ No ☐ ND

5.5 If yes, specify the number of hours from last milking to calving [i.e., 12, 24, 48, etc.] : _____ ☐ ND ☐ NA

5.6 If yes, enter code describing frequency of milking [see list of codes below]: _____

5.7 Is the dam being milked on the week after calving? ☐ Yes ☐ No ☐ ND

5.8 If yes, enter code describing frequency of milking [see list of codes below]: _____

5.9 After calving, has the dam been milked prior to the calf suckling colostrum for the first time? ☐ Yes ☐ No ☐ ND

1. Sporadically
2. < than once every two days
3. Once every two days
4. Once a day
5. Twice a day
6. Three times a day
7. Other [Specify]
8. ND
9. NA

6. Neonatal information [Tick as appropriate]

Was the calf able to suckled milk from the dam within 24 hours of birth? ☐ Yes ☐ No ☐ ND

If the calf was not able to suckle milk from the dam within 24 hours what did you do to make sure that the calf gets milk to drink?

1. Calf bottle fed colostrum from the same dam within 24 hours
2. Calf given artificially prepared colostrum within 24 hours ☐ Yes ☐ No ☐ NA
3. Calf been given colostrum from another dam within 24 hours ☐ Yes ☐ No ☐ NA

Umbilicus disinfected: ☐Yes ☐No ☐Not Sure / Not Done

7. Farmer Status

7.1 Level of Education:

☐ No formal education ☐ Adult literacy ☐ Primary school ☐ Secondary school ☐ Other: _____ ☐ ND

7.2 Technical training:

☐ Yes: University ☐ Yes: Other than university ☐ No ☐ ND

7.3 Position of farmer in household

☐ Husband/Wife ☐ Grandparent ☐ Son/Daughter ☐ Other: _____ ☐ ND

7.4 Tribe

☐ Samia ☐ Busuku ☐ Luo ☐ Teso ☐ Other: _____ ☐ ND

7.5 Occupation

☐ Farmer ☐ Teacher ☐ Civil Servant ☐ Business ☐ Retired with pension ☐ Retired no pension ☐ Other: _____ ☐ ND

7.6 Land Ownership:

☐ Owns land ☐ Owns and leases extra land ☐ Rents land ☐ Other: _____ ☐ ND

8. Crop types

8.1 Total Acres of land owned / rented by farmer: _____ ☐ ND ☐ NA

Total acres of land leased by farmer_____ ☐ ND ☐ NA

8.2 Farmer's Use of Land:

Main Crops	Acres	[ND]	Weight harvest (bags, Kg, Tones, Stacks)	[ND]
		☐		☐
		☐		☐
		☐		☐
		☐		☐
		☐		☐

Crops: 1= Banana, 2=Sugarcane,3=Sorghum,4= Finger-millet, 5=Maize, 6= Beans, 7=Cassava, 8=Sweet Potato, 9= Vegetables, 10=Irish potato / potato; 11=Cotton, 12=Rice, 13=Peanuts, 14=Tea, 15=Coffee, 16=Papyrus, 17=Groundnuts, 18=Tomatoes, 19= Napier Grass, 20=Tobacco, 21= Other (Specify)

9. Types and Numbers of Animals Kept by the Household

How many cattle do you keep in the household? [Enter the number kept as categorized by age group and sex in boxes provided]

Animal Types	**Total Numbers**		**Classification of Animal Types on the Basis of Age category and Sex**											
	Totals	[?]	Calves ♂	[?]	Calves ♀	[?]	Weaners ♂	ND	Weaners ♀	[?]	Adult ♂	[?]	Adults ♀	[?]
Indigenous cattle		☐		☐		☐		☐		☐		☐		☐
Grade cattle		☐		☐		☐		☐		☐		☐		☐
Crossed Cattle		☐		☐		☐		☐		☐		☐		☐
Other Spp														
Goats														
Sheep														
Pigs														
Dogs														

10. Animal Husbandry and Management

Ask an open question and tick the appropriate answer. Please note that **D = Dry Season and W = Wet Season**

Tick 'ND' [Not done / not sure] if you do not know the answer to a question.

1. How do you graze / Feed your cattle? Tick one option for each season.

	D	W
Herded	□	□
Paddock	□	□
Tethered	□	□
Stall fed	□	□
Yard	□	□
Free grazing	□	□
Other : _____	□	□
Not sure/Not done	□	□

2. How are your cattle housed? Tick one option for each season.

	D	W
Kraal	□	□
Stall / shed	□	□
Yard	□	□
None	□	□
Other: _____	□	□
Not sure/Not done	□	□

Calves grazed/fed with adults? □Yes □No □ND

Are calves housed with adults? □Yes □No □ND

3. What materials have you used for the cattle house? You may tick one or more options. No need to differentiate by season.

	Yes	No	ND	NA
Untreated wood / Bush	□	□	□	□
Treated wood	□	□	□	□
Thatch	□	□	□	□
Iron sheets	□	□	□	□
Bricks / Stone	□	□	□	□
Mud / Earth	□	□	□	□
Wire	□	□	□	□
Other:_____				
Other:_____				

4. Form of Housing: Tick one option per each section (i.e. Roof, solid wall, floor). No need to differentiate by season.

	Yes	No	ND	NA
Roof	□	□	□	□
Solid Wall	□	□	□	□
Floor:				
Concrete	□			
Wooden	□			
Earth	□			
ND	□			
NA	□			

5. Supplementation Regime: You may tick one or more options for each season.

	D	W
Roughage/crop residue	□	□
Minerals [salts] / vitamins	□	□
Bought-in feed/concentrates	□	□
None	□	□
Other:_____	□	□
Other:_____	□	□
Not sure/Not done	□	□

6. How cattle are watered: Tick one option for each season.

	D	W
Animals go to water	□	□
Water fetched/provided	□	□
Both	□	□
Not sure/Not done	□	□

7. Source of water: You may tick one or more options for each season.

	D	W
Borehole	□	□
Dam/Pond	□	□
River	□	□
Water well	□	□
Spring	□	□
Municipal/piped	□	□
Other:_____	□	□
Other:_____	□	□
Not sure/Not done	□	□

8. Distance to furthest watering point: Tick one option for each season.

	D	W
At household	□	□
<1 km	□	□
1-5 km	□	□
6-10 km	□	□
>10 km	□	□
Not sure/Not done	□	□

9. Frequency of Watering: Tick one option for each season.

	D	W
Freely available	□	□
Once a day	□	□
Twice a day	□	□
Every other day	□	□
Once in 3 days	□	□
Other:_____	□	□
Not sure/Not done	□	□

10. Water quality: Tick one option for each season.

	D	W
Good/clear	□	□
Muddy	□	□
Salty	□	□
Smelly	□	□
Not sure/Not done	□	□

11. Animal Health

1. Do you get access to veterinary services? □Yes □No □ND

If yes, where do you normally get access to veterinary services? You may tick one or more options.

Government veterinarian	□
Private veterinarian	□
Veterinary drug supplier	□
Extension service	□
Other:_____	□

3. Disease codes:
1 = Diarrhoea;
2 = Fever;
3 = Lack of appetite;
4 = Skin problems ;
5 = Swollen lymph nodes;
6 = Weight loss;
7 = Coughing;
8 = Sudden death;
9 = Swollen muscles;
10 = Helminthiasis;
11 = East Coast Fever (ECF);
12 = Foot and Mouth Disease (FMD);
13 = Calf mortality diseases;
14 = Respiratory conditions ;
15 = Lumpy Skin Disease (LSD) ;
16 = Anaplasmosis;
17 = Babesiosis;
18 = Anthrax;
19 = Heartwater;
20 = Trypanosomiasis;
21 = Tick Borne Diseases (TBDs);
22 = Hard Faeces;
23 = Abortions ;
24 = Infertility ;
25 = Mastitis ;
26 = Other (Specify)

2. Are there any diseases of cattle in the farm? □Yes □No □ND

If yes, list the cattle diseases or symptoms that often occur in the farm. Write disease code or name in space provided. The first three diseases should be ranked (i.e. most common first). Please try to get the generic name of the treatment rather than the brand. See last page for treatment codes.

List of Diseases/symptoms	Are animals treated if sick? Yes	No	ND	Generic Treatment
1. _____	□	□	□	1. _____
2. _____	□	□	□	2. _____
3. _____	□	□	□	3. _____
4. _____	□	□	□	4. _____
5. _____	□	□	□	5. _____
6 _____	□	□	□	6. _____
7. _____	□	□	□	7. _____
8. _____	□	□	□	8. _____
9. _____	□	□	□	9. _____
10. _____	□	□	□	10. _____

4. Are there any preventive treatments / Vaccinations for cattle? □Yes □No □ND

If yes, which vaccines do you use in your farm? You may tick one or more options. For each option you tick, specify whether the vaccine is given routinely (**R**) or whether is given only when need arises (**WN**). If you don't known the frequency tick '**ND**'. If you know that the farmer is vaccinating cattle but you don't know the type of vaccine tick 'Unknown vaccine '. You may enter as many unknown vaccines as needed.

	R	WN	ND
Muguga cocktail (ECF)	□	□	□
CBPP	□	□	□
FMD	□	□	□
LSD	□	□	□
BQ	□	□	□
Anthrax	□	□	□
Brucella	□	□	□
Rinderpest	□	□	□
Rift Valley Fever	□	□	□
Rabies	□	□	□
Heartwater	□	□	□
Anaplasmosis	□	□	□
Babesiosis	□	□	□
Haemorrhagic Septicemia	□	□	□
BVD	□	□	□
IBR	□	□	□

	R	WN	ND
BRSV	□	□	□
PI-3	□	□	□
Shipping Fever Complex	□	□	□
Leptospirosis	□	□	□
Rotavirus and Coronavirus	□	□	□
Colibacillosis	□	□	□
E. coli endotoxin	□	□	□
Salmonella	□	□	□
Pink Eye	□	□	□
Campilobacter	□	□	□
Haemophylus	□	□	□
Other:_____	□	□	□
Other:_____	□	□	□
Unknown vaccine 1	□	□	□
Unknown vaccine 2	□	□	□
Unknown vaccine 3	□	□	□

5. Do you control ectoparasites (ticks) in cattle? □Yes □No □ND

If yes, specify methods used

Method	Drug / Traditional Remedy Used	Frequency Dry Season	Wet Season
1. _____	_____	Every _____ weeks	Every _____ weeks
1. _____	_____	Every _____ weeks	Every _____ weeks
1. _____	_____	Every _____ weeks	Every _____ weeks
1. _____	_____	Every _____ weeks	Every _____ weeks

Codes for Method:

[1] Spraying of legs only
[2] Spraying of whole body
[3] Dipping
[4] Pour - on
[5] Hand dressing
[6] Injectables
[7] Traditional
[8] Other
[ND] Don't know

Codes for Drugs / Traditional remedies used : See Last Page for Treatment Codes

Codes for Frequency: Enter **'WN'** if treatment is only done when need arises; enter number of weeks if treatment is done routinely. Enter **'ND'** if you don't know the frequency of treatments.

11. Animal Health [Continued]

6. Do you control trypanosomiasis in cattle? □Yes □No □ND

If yes, specify methods used

Method	Drug / Traditional Remedy Used	Frequency: Dry Season	Frequency: Wet Season
1. _____	_____	Every _____ weeks	Every _____ weeks
1. _____	_____	Every _____ weeks	Every _____ weeks
1. _____	_____	Every _____ weeks	Every _____ weeks
1. _____	_____	Every _____ weeks	Every _____ weeks

Codes for Method:

[1] Spraying of legs only
[2] Spraying of whole body
[3] Dipping
[4] Pour - on
[5] Hand dressing
[6] Chemotherapy
[7] Traditional
[8] Other
[ND] Don't know

Codes for Drugs / Traditional remedies used : See Last Page for Treatment Codes

Codes for Frequency: Enter **'WN'** if treatment is only done when need arises; enter number of weeks if treatment is done routinely. Enter **'ND'** if you don't know the frequency of treatments.

7. Do you control intestinal parasites of cattle? □Yes □No □ND

If yes, specify methods used

Method	Drug / Traditional Remedy Used	Frequency: Dry Season	Frequency: Wet Season
1. _____	_____	Every _____ weeks	Every _____ weeks
1. _____	_____	Every _____ weeks	Every _____ weeks
1. _____	_____	Every _____ weeks	Every _____ weeks
1. _____	_____	Every _____ weeks	Every _____ weeks

Codes for Method:

[1] Drench
[2] Bollet
[3] Feed
[4] Injectable
[5] Paste
[6] Pour - on
[7] Salt Block
[8] Traditional
[9] Other
[ND] Don't know

Codes for Drugs / Traditional remedies used : See Last Page for Treatment Codes

Codes for Frequency: Enter **'WN'** if treatment is only done when need arises; enter number of weeks if treatment is done routinely. Enter **'ND'** if you don't know the frequency of treatments.

12. Entries

Ask an open question and tick the appropriate answer in the first box. Enter 'ND' [Not done / not sure] if the answer to the question is not known.

Market codes:

1=Myanga,2=Nasianda,3=Mateka,4=BGM,5=Mamboleo,6=Nawinyi,7=Bungoma,8=Sangalo,9=Mungatsi,10=Nambale,11=Amukura,12=Kimimini, 12=Kemodo, 13=Adungosi, 14=Bumula, 15=Asiriam, 16=Angorom,17=Bugengi,18=Bumala,19=Koloo,20=Funyula,21=Jera, 22=Nambale, 23=Boro
24= Bumala "B", 25= Lugulu, 26=Nyadorora, 26=Yala, 27=Simeriro, 28=Sigomere, 29=Mayanja, 30=Chwele

1. Where do you buy your cattle?

[1] Within sub-location ☐
[2] Neighbouring sub-location ☐ *SL Name [_____]*
[3]Livestock market ☐ *Market Code: [____]*

13. Entries

Ask an open question and tick the appropriate answer in the first box. Enter 'ND' [Not done / not sure] if the answer to the question is not known.

Breed codes:

1=Indigenous, 2=exotic , 3=cross.

1. How are you animals served when breeding and what breed of bull do you use? (You may enter one or more options)

	Tick	Specify: 1/2/3	Breed 1 (Common Name)	Breed 1 (Local Name)	Breed 2 (Common Name)	Breed 2 (Local Name)
Own bull (bred)	☐					
Own bull (bought)	☐					
Bull donated	☐					
Bull borrowed	☐					
Artificial insemination	☐					
Communal area bull	☐					
Not sure	☐					
Other (Specify):	☐					

1=Indigenous, 2=exotic , 3=cross.

ADDITIONAL INFORMATION

1. Was the dam born in the household? □ **Yes** □ **No** □ **ND**

2. Does the household have chickens? □ **Yes** □ **No** □ **ND**

If yes, specify number: _____

3. If yes: Are chickens kept together with the cattle? □ **Yes** □ **No** □ **ND**

At which time of day are chickens together with the cattle?

-Night only □
-Day only □
-Day and night □
-ND □

4. Specify other animals (besides those in Section 9 and chickens) kept in household: _____

5. Comments on grazing: If farmer practices free grazing, explain whether grazing is communal and whether the site for grazing is always the same or not (add any details **including GPS coordinates** if possible):

DRY SEASON:

_____

_____

WET SEASON:

_____

_____

6. Comments on watering: If the farmer takes animals to get water, explain whether water point is communal and whether the site is always the same or not (add any details **including GPS coordinates** if possible):

DRY SEASON:

_____

_____

WET SEASON:

_____

_____

7. Section 13, refers to common practices in the whole herd with regards to bulls used to serve the cows. Now answer specifically for the recruited calf:

How was the dam served when breeding? (Enter only one option)

	Tick	Specify: 1/2/3	Breed 1 (Common Name)	Breed 1 (Local Name)	Breed 2 (Common Name)	Breed 2 (Local Name)
Own bull (bred)	☐					
Own bull (bought)	☐					
Bull donated	☐					
Bull borrowed	☐					
Artificial insemination	☐					
Communal area bull	☐					
Not sure	☐					
Other (Specify):	☐					

1=Indigenous, 2=exotic , 3=cross.

MAIN HOUSEHOLD QUESTIONNAIRE: TREATMENT CODES

CATEGORY	CODE	DRUG
Unknown Category	41	Don't Know
	42	Other
Antihelminthic	1	Albendazole
	2	Ivermectin
	43	Clorsulon
	44	Thiabendazol
	45	Levamisole
	46	Morantel
	47	Fenbendazole
	48	Eprinomectin
	49	Moxidectin
	50	Doramectin
	51	Levamisole with Oxyclozaride
	3	Other
	4	Don't Know
Antibiotic	5	LA Oxytetracycline
	6	SA Oxytetracycline
	54	Penicillin (Procaine)
	55	Penicillin (Procaine - Benzathine)
	56	Erythromycin
	57	Tylosin
	58	Sulfadimethoxine
	59	Amoxicillin
	60	Ampicillin
	61	Ceftoifur
	62	Tilmicosin
	63	Florfenicol
	52	Tetracycline
	53	Chlortetracycline
	64	Amprolium
	65	Lasalocid
	66	Monensin
	7	Other
	8	Don't Know
Trypanocidal	9	Diminazene
	10	Isomethamidium
	11	Homidium Salt : Undefined Type
	68	Homidium Salt: Homidium Cloride
	69	Homidium Salt: Homidium Bromide
	71	Homidium Salt: Ethidium
	12	Other
	13	Don't Know
Antiprotozoal	14	Imidocarb
	15	Parvaquone
	16	Buparvaquone
	67	Toltrazuril
	17	Other
	18	Don't Know
Insecticide-Acaricide	19	Deltamethrone
	20	Other
	21	Don't Know
Traditional	22	Paraffin
	23	Tick Grease
	24	Manual Removal of Ticks
	25	Burning Lymph Nodes
	93	Medicinal Plants
	70	Walking on mud
	72	Burning cattle dung - Lightingh Fires
	26	Other
	27	Don't Know
Other	28	Topical Antiseptic
	29	Vitamin Supplement
Other	30	Other
	31	Don't Know
Vaccine	32	Muguga Cocktail
	33	CBPP
	34	FMD
	35	LSD
	36	BQ
	37	Anthrax
	38	Brucella
	73	Rinderpest
	74	Rift Valley Fever
	75	Rabies
	76	Heartwater
	77	Anaplasmosis
	78	Babesiosis
	79	Haemorrghagic Septicemia
	80	BVD
	81	IBR
	82	BRSV
	83	PI-3
	84	Shipping Fever Complex
	85	Leptospirosis
	86	Rotavirus and Coronavirus
	87	Colibacillosis
	88	E. coli endotoxin
	89	Salmonella
	90	Pink Eye
	91	Campylobacter
	92	Haemophylus
	39	Other
	40	Don't Know

Appendix D

Dam questionnaire

This was delivered at every 5-weekly routine visit up until the time the calf was weaned. It was also delivered if the calf died before weaning.

Clinical Examination of the Dam

1. Visit Details

Dam ID AHA / Vet ID

Visit ID Date: _____ Time: _____

Visit Type: ☐7d ☐Monthly ☐Final [Immediately after notice that the calf is no longer suckling milk from the dam].

2. Follow-Up History

1. Is the dam available to proceed with the visit?

☐ Yes ☐ No

If no, select one reason from below, and close this questionnaire.

☐ Unmanageable [Provisional loss]	☐ Slaughtered [Permanent Loss]	☐ Dead [Permanent loss]
☐ Out grazing [Provisional loss]	☐ Dowry [Permanent Loss]	☐ Other_____
☐ Sold [Permanent Loss]	☐ Stolen [Permanent Loss]	☐ Not Sure / Not Done [ND]

Detailed Clinical Examination

You should complete this section every time you visit the dam.

3. Subjective AHA / Veterinarian Assessment Dam's Health

Health Score: ☐**Apparently Well** ☐**Wounded** ☐**Sick** ☐**Dying** ☐**ND**

4. Condition Score

Score 1 to 9; In a borderline case add half point to the lower score:

☐ **1** ☐ **1-2** ☐ **2** ☐ **2-3** ☐ **3** ☐ **3-4** ☐ **4** ☐ **4-5** ☐ **5** ☐ **5-6** ☐ **6** ☐ **6-7** ☐ **7** ☐ **7-8** ☐ **8** ☐ **8-9** ☐ **9** ☐ **ND**

[See interpretation of condition scores below]

1 = L-	4 = M-	7 = F-
2 = L	5 = M	8 = F
3 = L+	6 = M+	9 = F+

5. General Anomalies of the Udder Region

☐ **Normal** ☐ **Abnormal** ☐ **ND**

If abnormal, tick one or more lesions below: For each lesion you tick, enter a digit to indicate the extend of the lesion as follows: 1 = Focal; 2 = Multi-focal; 3 = Diffuse; ND = Not Sure / Not Done.

☐ [1] Ventral Oedema [Pitting on Pressure] [ ]	☐ [3] Dropped Udder [Rupture of udder ligaments] [ ]	☐ ND [ ]
☐ [2] Ventral Haematoma [No Pitting on Pressure] [ ]	☐ [4] Other_____ [ ]	

6. Udder Lymph Nodes

☐ **Normal** ☐ **Abnormal** ☐ **ND**

If abnormal select lesions from list below. For each lesion you tick, enter 1 = Right; 2 = Left; 3 = Bilateral; ND = Not sure / not done:

☐ [1] Enlarged [Hyperplasic] [ ]	☐ [3] Fistula [ ]	☐ [5] Other: _____ [ ]
☐ [2] Atrophied [Smaller] [ ]	☐ [4] Abscess [ ]	☐ ND [ ]

7. Udder Lesions

	Normal	Abnormal	ND						
Front Udder [RL]	□	□	□	___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____	___[ ]:_____
				___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____	___[ ]:_____
				___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____	___[ ]:_____
				___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____	___[ ]:_____
				___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____	___[ ]:_____
Back Udder [RL]	□	□	□	___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____	___[ ]:_____
				___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____	___[ ]:_____
				___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____	___[ ]:_____
				___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____	___[ ]:_____
				___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____	___[ ]:_____
Front Teat [RL]	□	□	□	___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____	___[ ]:_____
				___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____	___[ ]:_____
				___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____	___[ ]:_____
				___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____	___[ ]:_____
				___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____	___[ ]:_____
Back Teat [RL]	□	□	□	___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____	___[ ]:_____
				___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____	___[ ]:_____
				___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____	___[ ]:_____
				___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____	___[ ]:_____
				___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____	___[ ]:_____
Front Udder Content [RL]	□	□	□	___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____	___[ ]:_____
				___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____	___[ ]:_____
				___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____	___[ ]:_____
				___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____	___[ ]:_____
				___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____	___[ ]:_____
Back Udder Content [RL]	□	□	□	___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____	___[ ]:_____
				___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____	___[ ]:_____
				___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____	___[ ]:_____
				___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____	___[ ]:_____
				___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____	___[ ]:_____

If abnormal, select from list below. You may enter any number of lesions for 'Udder & Teat' or 'Udder Content'. For each lesion that you tick, you must enter a **combined score**. The first digit on the score indicates the **location of the lesion**: 1 = Right; 2 = Left; 3 = Bilateral; 4 = Not sure, not done. The second digit in the score indicates **the extent of the lesion**: 1 = Focal ; 2 = Multi-focal; 3 = Diffuse; 4 = Mixture of 1 & 2 ; 5 = Mixture of 1 & 3; 6 = Mixture of 2 & 3; 7 = Not sure, not done; 8 = Not applicable in the case of anomalies of udder content. e.g. If you have multi-focal abscesses in the skin of the right front udder, and a single abscess in the skin of the left front udder, you would click 'Udder and Teat', then 'Abscesses' and you would enter code = 34.

Udder & Teat		Udder Content
[1] Pain [milking not possible]	[14] Scabs	[28] Blood clots
[2] Blind Udder [non functional]	[15] Fibrotic scars	[29] White [milk] clots
[3] Atrophied	[16] Warts	[30] Free floating rubbery masses
[4] Distended	[17] Necrosis / Gangrene	[31] Blood-tinged milk [pink milk]
[5] Hard Udder / Teat [granulomatous tissue]	[18] Bruising, haematomas	[32] Haemorrhagic milk [red milk]
[6] Swelling / oedema [pitting on pressure]	[19] Lacerations	[33] Port-wine secretion
[7] Hyperaemia/congestion	[20] Granulomas / nodules	[34] Watery translucent serous exudate
[8] Blisters [vesicles/pustules /papules]	[21] Abcesses	[35] Brownish serum-coloured exudate
[9] Non-exudative ulcers/erosions	[22] Foul odour/Malodour	[36] Homogeneous brown secretion
[10] Ulcers/erosions [serous exudates]	[23] Teat orifice stenosis	[37] Gas
[11] Ulcers/erosions [serofibrinous exudates]	[24] Obstruction of teat orifice	[38] Pus [yellow thick secretion]
[12] Ulcers/erosions [purulent exudates]	[25] Everted teat canal	[39] Foul odour/Malodour
[13] Ulcers/erosions [haemorrhagic exudates]	[26] Other: *[Max. 30characters and no space]*	[40] Other: *[Max. 30characters and no space]*
	[27] Not sure, not done	[41] Not sure, not done

8. Body Measurements

Girth _____ cm ☐ ND

9. Compulsory Sample Collection: Blood

Collection of blood samples as shown below is compulsory at the 7d visit and at the final visit [i.e. the final visit takes place as soon as you get notice of weaning]. Click 'NA' [not applicable] if this is a monthly visit. Click 'ND' [not done] if sample collection was applicable but not possible. You may collect a greater number of EDTA / Plain / Heparin vacutainers depending on the number of tests you want to perform.

Select types from list below and enter Barcode ID. The number of samples required is shown in brackets:

Jugular vein: Plain 10 ml vacutainer [N = 2 TUBES]:

Sample ID _____ Number of samples *2* ☐ ND ☐ NA

Jugular vein: EDTA 5 ml vacutainer [N = 1 TUBE]:

Sample ID _____ Number of samples *1* ☐ ND ☐ NA

Jugular vein: EDTA 10 ml vacutainer with Magic Buffer [N = 2 TUBES]:

Sample ID _____ Number of samples *2* ☐ ND ☐ NA

10. Compulsory Sample Collection: Milk

You should collect two milk samples **on the first monthly visit ONLY [VRC06]**. Tick 'ND' if milk collection was not possible at this visit. Tick 'NA' if this is not the first monthly visit.

Milk: [N = 2 TUBES ; One for biobank and one analysis]. Pull milk from all healthy quarters (i.e. those that do not present macroscopic signs of mastitis such as haemorrghagic milk, etc.)

Sample ID _____ Number of samples *2* ☐ ND ☐ NA

11. Pictures

It is compulsory that you take AT LEAST one picture from each lesion for later validation of the current classification of lesions. You must save your pictures electronically, but a paper copy of the pictures is also required. It is compulsory that you attach the PTC ID of your pictures here:

PTC ID [Attach Label]: _____

Description (Section, Body Part, Lesion Code, etc.):

_____

_____

_____

PTC ID [Attach Label]: _____

Description (Section, Body Part, Lesion Code, etc.):

_____

_____

_____

PTC ID [Attach Label]: _____

Description (Section, Body Part, Lesion Code, etc.):

_____

_____

_____

PTC ID [Attach Label]: _____

Description (Section, Body Part, Lesion Code, etc.):

_____

_____

_____

Appendix E

Routine visit questionnaire relating to the herd and calf health

This questionnaire was delivered at every 5-weekly routine visit. Apart from recording information from the farmer, it also guided the IDEAL staff through the clinical examination of the calf.

1. Visit Details

Calf ID AHA / Vet ID

Visit ID Date: _____ Time: _____

Visit Type: ☐7d Visit ☐*Weekly Visit* ☐Monthly Visit ☐Yearly Visit [VRC51] ☐Clinical Episode Visit [VCC]

2. Follow-Up History

1. Is the calf available to proceed with the visit?

☐ Yes ☐ No

If no, select one reason from below, and close this questionnaire. CALL VET & GO TO POST MORTEM FORM IF REASON FOR LOSS TO FOLLOW-UP IS DEATH OF CALF:

☐ Unmanageable [Provisional loss]	☐ Slaughtered [Permanent Loss]	☐ Dead [Permanent loss; GO TO POSTMORTEM FORM]
☐ Out grazing [Provisional loss]	☐ Dowry [Permanent Loss]	☐ Other_____ *[30characters and no space]*
☐ Sold [Permanent Loss]	☐ Stolen [Permanent Loss]	☐ Not Sure / Not Done [ND]

3. Inter-Visit History: Weaning and Grazing

1. Is the calf still suckling milk from the dam?

☐ Yes ☐ No ☐ Unknown

2. Does the calf go out grazing with the adults?

☐ Yes ☐ No ☐ Unknown

4. Inter-Visit History: Animal Movements, Mortality & Animal Bites

This section should only be completed at monthly and yearly visits. It should also be completed in clinical episode visits [VCC] and any routine visit where a clinical episode is detected. You will tick 'NA' [not applicable] if this is a 7D visit or a weekly visit with no clinical episode, and you will leave questions 1 – 2 blank.

NA ☐

1. Write down the number of NEW animals in the herd [In] and the numbers of animals that have left the herd [Out] since the last visit to the farm with inter-visit history. The latter includes losses due to deaths. Also record the number of animals currently in the herd [Current] and the animals that have died during the inter-visit period.

For each animal category, when recording the number of new animals, tick 'ND' if you don't know whether there have been entries in the herd. Tick '>0' if you know that there are new animals but you don't know how many. The same applies for 'OUT', 'CURRENT' and 'DEAD'. For the case of 'CURRENT' and 'DEAD', tick 'NA' if there were no such animals in the herd in the first place. In such case, the question would be 'Not Applicable'. Please note that you should also consider the dam and the calf as part of the herd. So you need to count these animals in the corresponding categories and when doing the total counts.

	IN			OUT				CURRENT			DEAD				REASONS FOR DEATH
	N	ND	> 0	N	ND	>0	NA	N	ND	> 0	N	ND	>0	NA	____ [ ] ____ [ ] ____ [ ] ____ [ ]
CBH-M		☐	☐		☐	☐	☐		☐	☐		☐	☐	☐	____ [ ] ____ [ ] ____ [ ] ____ [ ]
CBH-F		☐	☐		☐	☐	☐		☐	☐		☐	☐	☐	____ [ ] ____ [ ] ____ [ ] ____ [ ]
CNBH-M		☐	☐		☐	☐	☐		☐	☐		☐	☐	☐	____ [ ] ____ [ ] ____ [ ] ____ [ ]
CNBH-F		☐	☐		☐	☐	☐		☐	☐		☐	☐	☐	____ [ ] ____ [ ] ____ [ ] ____ [ ]
W-M		☐	☐		☐	☐	☐		☐	☐		☐	☐	☐	____ [ ] ____ [ ] ____ [ ] ____ [ ]
W-F		☐	☐		☐	☐	☐		☐	☐		☐	☐	☐	____ [ ] ____ [ ] ____ [ ] ____ [ ]
A-M		☐	☐		☐	☐	☐		☐	☐		☐	☐	☐	____ [ ] ____ [ ] ____ [ ] ____ [ ]
A-F		☐	☐		☐	☐	☐		☐	☐		☐	☐	☐	____ [ ] ____ [ ] ____ [ ] ____ [ ]
TOTAL		☐	☐		☐	☐	☐		☐	☐		☐	☐	☐	____ [ ] ____ [ ] ____ [ ] ____ [ ]

If there is a history of deaths, enter the reasons for death as follows: **1** = Trauma / Accident; **2** = Animal bite; **3** = Born dead / abortion; **4** = Dead during calving; **5** = Dead 48 h after birth for reasons other that 1 or 2; **6** = Dead > 48 h after birth for reasons other that 1 or 2; **ND** = Not done, don't know. For each reason, you must specify the number of affected animals [either a **number** or '**ND**' if you don't know how many animals died due to the specified reason].

2. Is there a history of animal bites between this visit and the previous [7D/monthly] visit?

If the dam is no longer in the herd, click 'NA'. If there are no other animals in the herd, click 'NA'.

	Yes	No	Unknown	NA	If yes, tick one or more options from list below.			
Calf	☐	☐	☐		☐ Dog bite	☐ Snake bite	☐ Other_____	☐ Don't know
Dam	☐	☐	☐	☐	☐ Dog bite	☐ Snake bite	☐ Other_____	☐ Don't know
Other animals in herd	☐	☐	☐	☐	☐ Dog bite	☐ Snake bite	☐ Other_____	☐ Don't know

Abbreviations: CBH-M/F = 'Male/Female calves born in household'; CNBH-M/F = 'Male/Female calves not borne in household'; W-M/F = 'Male/female weaners; A-M/F = 'Male/Female adults'.

5. Inter-Visit History: Veterinary Interventions

This section should only be completed at monthly and yearly visits. It should also be completed in clinical episode visits [VCC] and any routine visit where a clinical episode is detected. You will click 'NA' [not applicable] if this is a 7D visit or a weekly visit with no clinical episode, and you will leave the questions blank.

NA □

1. Is there a history of veterinary interventions between this visit and the last visit with inter-visit history?
For the case of the dam, and for the case of other animals in the herd, you will tick 'NA' [not applicable] if the dam is no longer in the farm [i.e if sold, dead, etc.] or if there are no other animals in the herd.

If yes, select the type/s of treatment from below. In the case of 'other animals in the herd', for each treatment you tick, enter the number of treated animals. If you are not sure about the exact number of treated animals, enter one of the following scores: **A** = Up to 10% of the animals; **B** = Approximately > 10% but < 50% of the animals. **C =** Approximately 50% of the animals [~50%]; **D** = Most animals [> 50% herd]; **E** = All animals in herd; **ND** = Don't know. For the case of other animals in the herd, if you enter the number of treated animals, make sure that you have entered the 'current number of animals in the herd' in the previous section. For the case of Insecticides / acaricides, for each product you enter, you must enter a code to indicate the type of application as follows: **1** = spraying of legs only; **2** = spraying of whole body; **3** = Dipping; **4** = Pour on; **ND** = Don't know.

Calf	**Dam**	**Other animals in the herd**
□Yes □No □ND [Not Sure/Not done]	**□Yes □No □ND [Not Sure/Not done] □NA**	**□Yes □No □ND [Not Sure/Not done] □NA**
If yes, select from list below:	*If yes, select from list below:*	*If yes, select from list below:*
Antihelminthics **□Yes**	Antihelminthics **□Yes**	Antihelminthics **□Yes**
□ Albendazole	□ Albendazole	□ Albendazole N ___
□ Ivermectin	□ Ivermectin	□ Ivermectin N ___
□ Other_____	□ Other_____	□ Other_____ N ___
□ Not Sure, don't know	□ Not Sure, don't know	□ Not Sure, don't know N ___
Antibiotics **□Yes**	Antibiotics **□Yes**	Antibiotics **□Yes**
□ LA oxytetracycline	□ LA oxytetracycline	□ LA oxytetracycline N ___
□ SA oxytetracycline	□ SA oxytetracycline	□ SA oxytetracycline N ___
□ Other_____	□ Other_____	□ Other_____ N ___
□ Not Sure, don't know	□ Not Sure, don't know	□ Not Sure, don't know N ___
Trypanocidals **□Yes**	Trypanocidals **□Yes**	Trypanocidals **□Yes**
□ Diminazene	□ Diminazene	□ Diminazene N ___
□ Isomethamidium	□ Isomethamidium	□ Isomethamidium N ___
□ Homidium salts	□ Homidium salts	□ Homidium salts N ___
□ Other_____	□ Other_____	□ Other_____ N ___
□ Not Sure, don't know	□ Not Sure, don't know	□ Not Sure, don't know N ___
Antiprotozoals **□Yes**	Antiprotozoals **□Yes**	Antiprotozoals **□Yes**
□ Imidocarb	□ Imidocarb	□ Imidocarb N ___
□ Parvaquone	□ Parvaquone	□ Parvaquone N ___
□ Buparvaquone	□ Buparvaquone	□ Buparvaquone N ___
□ Other_____	□ Other_____	□ Other_____ N ___
□ Not Sure, don't know	□ Not Sure, don't know	□ Not Sure, don't know N ___
Insecticides/Acaricides **□Yes**	Insecticides/Acaricides **□Yes**	Insecticides/Acaricides **□Yes**
□ Deltamethrone A [ ]	□ Deltamethrone A [ ]	□ Deltamethrone A [ ] N ___
□ Other_____ A [ ]	□ Other_____ A [ ]	□ Other_____ A [ ] N ___
□ Not Sure, don't know A [ ]	□ Not Sure, don't know A [ ]	□ Not Sure, don't know A [ ] N ___
Traditional **□Yes**	Traditional **□Yes**	Traditional **□Yes**
□ Paraffin	□ Paraffin	□ Paraffin N ___
□ Tick grease	□ Tick grease	□ Tick grease N ___
□ Manual removal of ticks	□ Manual removal of ticks	□ Manual removal of ticks N ___
□ Burning of lymph nodes	□ Burning of lymph nodes	□ Burning of lymph nodes N ___
□ Other_____	□ Other_____	□ Other_____ N ___
□ Not Sure, don't know	□ Not Sure, don't know	□ Not Sure, don't know N ___
Other **□Yes**	Other **□Yes**	Other **□Yes**
□ Topical Antiseptic	□ Topical Antiseptic	□ Topical Antiseptic N ___
□ Vitamin supplementation	□ Vitamin supplementation	□ Vitamin supplementation N ___
□ Other_____	□ Other_____	□ Other_____ N ___
□ Not Sure, don't know	□ Not Sure, don't know	□ Not Sure, don't know N ___
Vaccines **□Yes**	Vaccines **□Yes**	Vaccines **□Yes**
□ Muguga cocktail	□ Muguga cocktail	□ Muguga cocktail N ___
□ CBPP	□ CBPP	□ CBPP N ___
□ FMD	□ FMD	□ FMD N ___
□ LSD	□ LSD	□ LSD N ___
□ BQ	□ BQ	□ BQ N ___
□ Anthrax	□ Anthrax	□ Anthrax N ___
□ Brucella	□ Brucella	□ Brucella N ___
□ Other_____	□ Other_____	□ Other_____ N ___
□ Not Sure, don't know	□ Not Sure, don't know	□ Not Sure, don't know N ___
Unknown Category **□Yes**	Unknown Category **□Yes**	Unknown Category **□Yes**
□ Other_____	□ Other_____	□ Other_____ N ___
□ Not Sure, don't know	□ Not Sure, don't know	□ Not Sure, don't know N ___

6. Inter-visit history & Inspection at Rest: Herd Health

This section should only be completed at monthly and yearly visits. It should also be completed in clinical episode visits [VCC] and any routine visit where a clinical episode is detected. You will click 'NA' [not applicable] if this is a 7D visit or a weekly visit with no clinical episode and you will leave the questions blank. You will also click 'NA' if there are no animals in the herd other than the calf of interest [i.e. no dam and no other animals

NA □

For each disorder present in the herd, you must enter a combined score. The first digit indicates **who has seen the lesions**: Enter **1** if you observe the disorder and enter **2** if you can't see the disorder, but the farmer has observed the disorder during the inter-visits time. The second digit in the score indicates **whether the dam is affected**: **1** = yes / **2** = no / **3** = don't know / **4** = The dam is no longer in the herd. The third digit in the score indicates the **extent of the problem at the herd level** [excluding the dam]: **1** = No affected animals; **2** = Up to 10% of the animals; **3** = Approximately > 10% but < 50% of the animals. **4** = Approximately 50% of the animals [~50%]; **5** = Most animals [> 50% herd]; **6** = All animals in herd; **7** = Don't know; **8** = There are no other animals in the herd.

e.g. If the farmer has observed a disorder for the case of the dam and all other animals in herd, which you have not seen during the visit, the code is: 216.

Feeding/Drinking

□ **Normal** □ **Abnormal** □ **Unknown**

If abnormal select from below:

□ Unable to swallow food [ ]
□ Food apprehension [ ]
□ Anorexia [ ]
□ Decreased appetite [ ]
□ Increased water intake [ ]
□ Decreased water intake [ ]
□ Other_____[ ]
□ Not sure / Not done [ ]

Mouth

□ **Normal** □ **Abnormal** □ **Unknown**

If abnormal select from below:

□ Hyperaemia [ ]
□ Haemorrhages [ ]
□ Blisters [Vesicles / Pustules] [ ]
□ Ulcers/erosions [ ]
□ Scabs / Scars [ ]
□ Other_____[ ]
□ Not sure / Not done [ ]

Feet

□ **Normal** □ **Abnormal** □ **Unknown**

If abnormal select from below:

□ Hyperaemia [ ]
□ Haemorrhages [ ]
□ Blisters [Vesicles / Pustules] [ ]
□ Ulcers/erosions [ ]
□ Scabs / Scars [ ]
□ Other_____[ ]
□ Not sure / Not done [ ]

Skin/Coat

□ **Normal** □ **Abnormal** □ **Unknown**

If abnormal select from below:

□ Generalised alopecia [ ]
□ Nodular lesions [ ]
□ Generalised sloughing [ ]
□ Excessive Sweating [ ]
□ Ulcers / Erosions [ ]
□ Scars / Scabs [ ]
□ Other_____[ ]
□ Not sure / Not done [ ]

Eyes

□ **Normal** □ **Abnormal** □ **Unknown**

If abnormal select from below:

□ Ocular Discharge [ ]
□ Corneal Opacity [ ]
□ Blindness [not nervous condition] [ ]
□ Other_____[ ]
□ Not sure / Not done [ ]

Posture

□ **Normal** □ **Abnormal** □ **Unknown**

If abnormal select from below:

□ Arched back [ ]
□ Recumbency [ ]
□ Extended head and neck [ ]
□ Star-gazing [ ]
□ Wide-based stance [ ]
□ Dog-sitting [ ]
□ Lateral positioning of head [ ]
□ Other_____[ ]
□ Not sure / Not done [ ]

Gait

□ **Normal** □ **Abnormal** □ **Unknown**

If abnormal select from below:

□ Lameness [ ]
□ Stiffness [ ]
□ Limping [ ]
□ Swaying hind quarter [ ]
□ Other_____[ ]
□ Not sure / Not done [ ]

Swelling

□ **Normal** □ **Abnormal** □ **Unknown**

If abnormal select from below:

□ Large muscle groups [ ]
□ Joints [ ]
□ Lymph nodes [ ]
□ Ventral thorax [ ]
□ Ventral abdomen [ ]
□ Other_____[ ]
□ Not sure / Not done [ ]

Nervous / Behaviour Change

□ **Normal** □ **Abnormal** □ **Unknown**

If abnormal select from below:

□ Nystagmus [ ]
□ Blindness [nervous condition] [ ]
□ Muscular tremors / muscle twitching [ ]
□ Convulsions [ ]
□ Incoordination / ataxia [ ]
□ High stepping gait [ ]
□ Circling [ ]
□ General weakness [ ]
□ Reduced sensitivity [ ]
□ Paralysis [ ]
□ Hypersensitivity [ ]
□ Restlessness [ ]
□ Lethargy [ ]
□ Aggression [ ]
□ Excessive licking [ ]

Nervous / Behaviour Change

□ Excessive chewing [ ]
□ Excessive salivation/drooling [ ]
□ Excessive bellowing [ ]
□ Other_____[ ]
□ Not sure / Not done [ ]

Respiratory

□ **Normal** □ **Abnormal** □ **Unknown**

If abnormal select from below:

□ Non-foamy nasal discharge [ ]
□ Foamy nasal discharge [ ]
□ Cough [ ]
□ Costo-abdominal respiration [ ]
□ Shallow / rapid breathing [ ]
□ Deep / laboured breathing [ ]
□ Other_____[ ]
□ Not sure / Not done [ ]

Gastrointestinal

□ **Normal** □ **Abnormal** □ **Unknown**

If abnormal select from below:

□ Tenesmus [straining] [ ]
□ Constipation [ ]
□ Hard faeces: Not bloody [ ]
□ Hard faeces: Bloody [ ]
□ Soiling [ ]
□ Diarrhoea: Not bloody [ ]
□ Diarrhoea: Bloody [ ]
□ Regurgitation / Vomiting [ ]
□ Other_____[ ]
□ Not sure / Not done [ ]

Urinary

□ **Normal** □ **Abnormal** □ **Unknown**

If abnormal select from below:

□ Straining [ ]
□ Excessive urination [ ]
□ Water coloured urine [ ]
□ Reddish-tinged urine [ ]
□ Brownish-tinged urine [ ]
□ Increased consistency of urine [>>density] [ ]
□ Other_____[ ]
□ Not sure / Not done [ ]

Ill Thrift

□ **Normal** □ **Abnormal** □ **Unknown**

If abnormal select from below:

□ Weight loss / loss of condition/poor condition [ ]
□ Cachexy / extreme thinness [ ]
□ Pale mucous membranes [ ]
□ Icterus [ ]
□ Cyanosis [ ]
□ Rough/staring coat. Not bright/shiny [ ]
□ Weakness [not nervous condition] [ ]
□ Other_____[ ]
□ Not sure / Not done [ ]

7. Inter-visit history & Inspection at Rest: Calf Health

This section will be completed EVERY TIME you visit the calf. For each disorder you tick, enter **1** if you have observed the disorder when inspecting the animal at rest and enter **2** if you have not observed the disorder, but the farmer has observed such disorder during the inter-visit history.

Feeding/Drinking

□ **Normal** □ **Abnormal** □ **Unknown**

If abnormal select from below:

□ Unable to swallow food [ ]
□ Food apprehension [ ]
□ Anorexia [ ]
□ Decreased appetite [ ]
□ Increased water intake [ ]
□ Decreased water intake [ ]
□ Other_____[ ]
□ Not sure / Not done [ ]

Mouth

□ **Normal** □ **Abnormal** □ **Unknown**

If abnormal select from below:

□ Hyperaemia [ ]
□ Haemorrhages [ ]
□ Blisters [Vesicles / Pustules] [ ]
□ Ulcers/erosions [ ]
□ Scabs / Scars [ ]
□ Other_____[ ]
□ Not sure / Not done [ ]

Feet

□ **Normal** □ **Abnormal** □ **Unknown**

If abnormal select from below:

□ Hyperaemia [ ]
□ Haemorrhages [ ]
□ Blisters [Vesicles / Pustules] [ ]
□ Ulcers/erosions [ ]
□ Scabs / Scars [ ]
□ Other_____[ ]
□ Not sure / Not done [ ]

Skin/Coat

□ **Normal** □ **Abnormal** □ **Unknown**

If abnormal select from below:

□ Generalised alopecia [ ]
□ Nodular lesions [ ]
□ Generalised sloughing [ ]
□ Excessive Sweating [ ]
□ Ulcers / Erosions [ ]
□ Scars / Scabs [ ]
□ Other_____[ ]
□ Not sure / Not done [ ]

Eyes

□ **Normal** □ **Abnormal** □ **Unknown**

If abnormal select from below:

□ Ocular Discharge [ ]
□ Corneal Opacity [ ]
□ Blindness [not nervous condition] [ ]
□ Other_____[ ]
□ Not sure / Not done [ ]

Posture

□ **Normal** □ **Abnormal** □ **Unknown**

If abnormal select from below:

□ Arched back [ ]
□ Recumbency [ ]
□ Extended head and neck [ ]
□ Star-gazing [ ]
□ Wide-based stance [ ]
□ Dog-sitting [ ]
□ Lateral positioning of head [ ]
□ Other_____[ ]
□ Not sure / Not done [ ]

Gait

□ **Normal** □ **Abnormal** □ **Unknown**

If abnormal select from below:

□ Lameness [ ]
□ Stiffness [ ]
□ Limping [ ]
□ Swaying hind quarter [ ]
□ Other_____[ ]
□ Not sure / Not done [ ]

Swelling

□ **Normal** □ **Abnormal** □ **Unknown**

If abnormal select from below:

□ Large muscle groups [ ]
□ Joints [ ]
□ Lymph nodes [ ]
□ Ventral thorax [ ]
□ Ventral abdomen [ ]
□ Other_____[ ]
□ Not sure / Not done [ ]

Nervous / Behaviour Change

□ **Normal** □ **Abnormal** □ **Unknown**

If abnormal select from below:

□ Nystagmus [ ]
□ Blindness [nervous condition] [ ]
□ Muscular tremors / muscle twitching [ ]
□ Convulsions [ ]
□ Incoordination / ataxia [ ]
□ High stepping gait [ ]
□ Circling [ ]
□ General weakness [ ]
□ Reduced sensitivity [ ]
□ Paralysis [ ]
□ Hypersensitivity [ ]
□ Restlessness [ ]
□ Lethargy [ ]
□ Aggression [ ]
□ Excessive licking [ ]

Nervous / Behaviour Change

□ Excessive chewing [ ]
□ Excessive salivation/drooling [ ]
□ Excessive bellowing [ ]
□ Other_____[ ]
□ Not sure / Not done [ ]

Respiratory

□ **Normal** □ **Abnormal** □ **Unknown**

If abnormal select from below:

□ Non-foamy nasal discharge [ ]
□ Foamy nasal discharge [ ]
□ Cough [ ]
□ Costo-abdominal respiration [ ]
□ Shallow / rapid breathing [ ]
□ Deep / laboured breathing [ ]
□ Other_____[ ]
□ Not sure / Not done [ ]

Gastrointestinal

□ **Normal** □ **Abnormal** □ **Unknown**

If abnormal select from below:

□ Tenesmus [straining] [ ]
□ Constipation [ ]
□ Hard faeces: Not bloody [ ]
□ Hard faeces: Bloody [ ]
□ Soiling [ ]
□ Diarrhoea: Not bloody [ ]
□ Diarrhoea: Bloody [ ]
□ Regurgitation / Vomiting [ ]
□ Other_____[ ]
□ Not sure / Not done [ ]

Urinary

□ **Normal** □ **Abnormal** □ **Unknown**

If abnormal select from below:

□ Straining [ ]
□ Excessive urination [ ]
□ Water coloured urine [ ]
□ Reddish-tinged urine [ ]
□ Brownish-tinged urine [ ]
□ Increased consistency of urine [>>density] [ ]
□ Other_____[ ]
□ Not sure / Not done [ ]

Ill Thrift

□ **Normal** □ **Abnormal** □ **Unknown**

If abnormal select from below:

□ Weight loss / loss of condition [ ]
□ Cachexy / extreme thinness [ ]
□ Pale mucous membranes [ ]
□ Icterus [ ]
□ Cyanosis [ ]
□ Rough/staring coat. Not bright/shiny [ ]
□ Weakness [not nervous condition] [ ]
□ Other_____[ ]
□ Not sure / Not done [ ]

Detailed Clinical Examination of the Calf and Compulsory Sample Collection

You should complete this section every time you visit the calf.

8. Rectal Temperature

	° C	☐ ND	*Repeat temperature reading if T ≤ 38.0 ° C*

9. Famacha Score

Right Eye: ☐ **<1** ☐ **1** ☐ **2** ☐ **3** ☐ **4** ☐ **5** ☐ **ND** *<1 [~ Conjunctivitis]*

Left Eye: ☐ **<1** ☐ **1** ☐ **2** ☐ **3** ☐ **4** ☐ **5** ☐ **ND** *<1 [~ Conjunctivitis]*

When in doubt between two scores, score the animal at the paler category

10. Neck-Skin Elasticity

☐ **Normal** ☐ **Poor** ☐ **Very Poor** ☐ **ND**

11. Lymph Nodes

	Normal	Abnormal	ND					
Parotid Lymph node	☐	☐	☐	___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____
Supra-scapular lymph node	☐	☐	☐	___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____
Pre-crural lymph node	☐	☐	☐	___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____

If abnormal select lesions from list below. For each lesion you tick, enter 1 = Right; 2 = Left; 3 = Bilateral; ND = Not sure / not done:

[1] Hyperplasic	[2] Atrophied	[3] Fistula	[4] Abscess	[5] Other: *[Max. 30characters no space]*	[ND] Not sure / not done

12. Calliper measures

Supra-scapular	**Right horizontal**		cm	☐ ND
	Left horizontal		cm	☐ ND
Pre-Crural	**Right horizontal**		cm	☐ ND
	Left horizontal		cm	☐ ND

13. Natural Openings: Discharge and Exudates

	None	Mild	Profuse	ND	NA					
Ocular[RL]	☐	☐	☐	☐		___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____
Nasal[RL]	☐	☐	☐	☐		___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____
Ear[RL]	☐	☐	☐	☐		___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____
Umbilicus	☐	☐	☐	☐		___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____
Udder	☐	☐	☐	☐	☐	___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____
Vaginal	☐	☐	☐	☐	☐	___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____
Prepucial	☐	☐	☐	☐	☐	___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____

If abnormal select type of discharge from list below [code]. For each lesion you select, enter 1 = Right; 2 = Left; 3 = Bilateral; 4 = Not applicable because the body part is not bilateral; ND = Not sure / not done:

[1] Serous discharge	[5] Purulent discharge	[9] Free blood
[2] Serofibrinous discharge	[6] Blood-tinged discharge	[10] Other: *[Max. 30characters and no space]*
[3] Mucus discharge	[7] Clear foam	[ND] Not sure, not done
[4] Mucopurulent discharge	[8] Blood-tinged foamy	

Normally, discharge types vary across natural openings as follows: Serofibrinous [applies to nasal, ocular]; Mucus or mucopurulent [applies to ocular / nasal/ vaginal]; Purulent discharge [applies to ear/nasal/navel/udder/vagina]; foamy [applies to nasal].

14. Skin, Mucous Membranes, Muscles, Abdomen, Bones, Hooves and Special Organs

	Normal	Abnormal	ND						
Face [RL]	□	□	□	___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] : _____	___ [ ] : _____
Head [not face; RL]	□	□	□	___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] : _____	___ [ ] : _____
Ear [RL]	□	□	□	___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] : _____	___ [ ] : _____
Eye ball [RL]	□	□	□	___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] : _____	___ [ ] : _____
Conjunctiva [RL]	□	□	□	___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] : _____	___ [ ] : _____
Cornea [RL]	□	□	□	___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] : _____	___ [ ] : _____
Sclera [RL]	□	□	□	___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] : _____	___ [ ] : _____
Eyelid [RL]	□	□	□	___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] : _____	___ [ ] : _____
Third eyelid [RL]	□	□	□	___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] : _____	___ [ ] : _____
Muzzle	□	□	□	___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] : _____	___ [ ] : _____
Nostrils [RL]	□	□	□	___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] : _____	___ [ ] : _____
Lips [DV]	□	□	□	___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] : _____	___ [ ] : _____
Gums [DV]	□	□	□	___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] : _____	___ [ ] : _____
Palate	□	□	□	___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] : _____	___ [ ] : _____
Tongue [DV]	□	□	□	___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] : _____	___ [ ] : _____
Mouth floor [interior]	□	□	□	___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] : _____	___ [ ] : _____
Cheeks [interior; RL]	□	□	□	___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] : _____	___ [ ] : _____
Inter-mandibular sp. [ext.]	□	□	□	___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] : _____	___ [ ] : _____
Neck [DV]	□	□	□	___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] : _____	___ [ ] : _____
Lateral neck [RL]	□	□	□	___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] : _____	___ [ ] : _____
Forelimb [RL]	□	□	□	___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] : _____	___ [ ] : _____
				___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] : _____	___ [ ] : _____
				___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] : _____	___ [ ] : _____
				___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] : _____	___ [ ] : _____
				___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] : _____	___ [ ] : _____

If abnormal, select from list below. For each body part you may select as many skin lesions / Muscular lesions / Bones, Hooves lesions / Special Organ lesions as you see. For each lesion that you enter you must enter a combined score. The first digit on the score indicates the **location of the lesion**: 1 = Right or dorsal / 2 = Left or ventral; 3 = Bilateral [Both RL or DV]; 4 = Not applicable because the body part is not bilateral; 5 = Not sure, not done. The second digit in the score indicates the **extent of the lesion**: 1 = Focal ; 2 = Multi-focal; 3 = Diffuse; 4 = Mixture of 1 & 2 ; 5 = Mixture of 1 & 3; 6 = Mixture of 2 & 3; 7 = Not sure, not done. e.g. If you have multi-focal abscesses in the skin of the right forelimb, and a single abscess in the skin of the left forelimb, you would click 'Skin', then 'Abscesses' and you would enter code = 34.

Skin & Mucous Membranes	[27] Fibrotic scars	[53] Fluid accumulation
[1] Alopecia	[28] Crusts	[54] Gas accumulation
[2] Sloughing	[29] Necrosis	[55] Abnormal convex shape causing asymmetry
[3] Pruritus	[30] Gangrene [not in mucous membranes]	[56] Abnormal concave shape causing asymmetry
[4] Swelling/oedema	[31] Foul-odour/Malodour	[57] Indurations
[5] Dry/scaly appearance [Hyperkeratosis]	[32] Abscesses	[58] Other: *[Max. 30characters and no space]*
[6] Pallor [Pale]	[33] Cracks	[59] Not Sure / Not Done
[7] Hyperaemia/congestion	[34] Loss of papillae [dorsal tongue only]	Bones & Hooves
[8] Icterus [yellowish]	[35] Foreign bodies	[60] Fracture / Fisures
[9] Cyanosis [bluish]	[36] Opacity [Cornea]	[61] Thickening
[10] Haemorrhages	[37] Perforation	[62] Stress lines [applies to hooves]
[11] Non-exudative nodules/granules	[38] Prolapse [a]	[63] White line disorder: Serum tinged [soles only]
[12] Exudative nodules/granules	[39] Other: *[Max. 30characters no space]*	[64] White line disorder: Blood tinged [soles only]
[13] Non-exudative raised clumps of hair	[40] Not Sure / Not Done	[65] Abnormal convex shape causing asymmetry
[14] Exudative raised clumps of hair	Muscles & Abdomen	[66] Abnormal concave shape causing asymmetry
[15] 'Bleeding spots'	[41] Pain	[67] Grown out
[16] 'Breathing holes' [warbles]	[42] Swelling/oedema/enlargement	[68] Other: *[Max. 30characters and no space]*
[17] Myasis	[43] Atrophy [smaller than normal]	[69] Not Sure / Not Done
[18] Other macroscopic ectoparasites	[44] Gangrene	Special Organs: Eye Balls
[19] Blisters [vesicles/ pustules/papules]	[45] Nodules	[70] Sunken
[20] Warts	[46] Abscesses	[71] Protruding
[21] Non-exudative ulcers/erosions	[47] Scabs	[72] Blepharospasm [closed eye due to pain]
[22] Ulcers/erosions [serous exudates]	[48] Fibrotic scars	[73] Internal haemorrhages
[23] Ulcers/erosions [serofibrinous exudates]	[49] Fistula [serous exudates]	[74] Cataract
[24] Ulcers/erosions [purulent exudates]	[50] Fistula [serofibrinous exudates]	[75] Thelazia
[25] Ulcers/erosions [haemorrhagic exudates]	[51] Fistula [purulent exudates]	[76] Other: *[Max. 30characters and no space]*
[26] Scabs	[52] Fistula [haemorrhagic exudates]	[77] Not Sure / Not Done

[a] Refers to third eyelid, rectum, vagina, prepuce [penis]…

14. Skin, Mucous Membranes, Muscles, Abdomen, Bones, Hooves and Special Organs [Continued]

	Normal	Abnormal	ND	NA						
Cor band forelimb [RL]	□	□	□		___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] :_____	___ [ ] :_____
Hoof forelimb [RL]	□	□	□		___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] :_____	___ [ ] :_____
Inter-digital forelimb [RL]	□	□	□		___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] :_____	___ [ ] :_____
Sole forelimb [RL]	□	□	□		___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] :_____	___ [ ] :_____
Thorax [DV]	□	□	□		___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] :_____	___ [ ] :_____
Lateral thorax [RL]	□	□	□		___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] :_____	___ [ ] :_____
Abdomen [DV]	□	□	□		___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] :_____	___ [ ] :_____
Umbilicus	□	□	□		___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] :_____	___ [ ] :_____
Udder	□	□	□	□	___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] :_____	___ [ ] :_____
Testis [RL]	□	□	□	□	___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] :_____	___ [ ] :_____
Penis	□	□	□	□	___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] :_____	___ [ ] :_____
Lateral abdomen [RL]	□	□	□		___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] :_____	___ [ ] :_____
Hindlimb [RL]	□	□	□		___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] :_____	___ [ ] :_____
Cor band hindlimb [RL]	□	□	□		___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] :_____	___ [ ] :_____
Hoof hindlimb [RL]	□	□	□		___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] :_____	___ [ ] :_____
Inter-digital hindlimb [RL]	□	□	□		___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] :_____	___ [ ] :_____
Sole hindlimb [RL]	□	□	□		___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] :_____	___ [ ] :_____
Perianal area	□	□	□		___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] :_____	___ [ ] :_____
Anus	□	□	□		___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] :_____	___ [ ] :_____
Vulva	□	□	□	□	___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] :_____	___ [ ] :_____
Tail	□	□	□		___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] :_____	___ [ ] :_____
	□	□	□		___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] :_____	___ [ ] :_____
					___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] :_____	___ [ ] :_____
					___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] :_____	___ [ ] :_____
					___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] :_____	___ [ ] :_____

If abnormal, select from list below. For each body part you may select as many skin lesions / Muscular lesions / Bones, Hooves lesions / Special Organ lesions as you see. For each lesion that you enter you must enter a combined score. The first digit on the score indicates the **location of the lesion**: 1 = Right or dorsal / 2 = Left or ventral; 3 = Bilateral [Both RL or DV]; 4 = Not applicable because the body part is not bilateral; 5 = Not sure, not done. The second digit in the score indicates the **extent of the lesion**: 1 = Focal ; 2 = Multi-focal; 3 = Diffuse; 4 = Mixture of 1 & 2 ; 5 = Mixture of 1 & 3; 6 = Mixture of 2 & 3; 7 = Not sure, not done. e.g. If you have multi-focal abscesses in the skin of the right forelimb, and a single abscess in the skin of the left forelimb, you would click 'Skin', then 'Abscesses' and you would enter code = 34.

Skin & Mucous Membranes

[1] Alopecia
[2] Sloughing
[3] Pruritus
[4] Swelling/oedema
[5] Dry/scaly appearance [Hyperkeratosis]
[6] Pallor [Pale]
[7] Hyperaemia/congestion
[8] Icterus [yellowish]
[9] Cyanosis [bluish]
[10] Haemorrhages
[11] Non-exudative nodules/granules
[12] Exudative nodules/granules
[13] Non-exudative raised clumps of hair
[14] Exudative raised clumps of hair
[15] 'Bleeding spots'
[16] 'Breathing holes' [warbles]
[17] Myasis
[18] Other macroscopic ectoparasites
[19] Blisters [vesicles/ pustules/papules]
[20] Warts
[21] Non-exudative ulcers/erosions
[22] Ulcers/erosions [serous exudates]
[23] Ulcers/erosions [serofibrinous exudates]
[24] Ulcers/erosions [purulent exudates]
[25] Ulcers/erosions [haemorrhagic exudates]
[26] Scabs
[27] Fibrotic scars
[28] Crusts
[29] Necrosis
[30] Gangrene [not in mucous membranes]
[31] Foul-odour/Malodour
[32] Abscesses
[33] Cracks
[34] Loss of papillae [dorsal tongue only]
[35] Foreign bodies
[36] Opacity [Cornea]
[37] Perforation
[38] Prolapse [a]
[39] Other: *[Max. 30characters no space]*
[40] Not Sure / Not Done

Muscles & Abdomen

[41] Pain
[42] Swelling/oedema/enlargement
[43] Atrophy [smaller than normal]
[44] Gangrene
[45] Nodules
[46] Abscesses
[47] Scabs
[48] Fibrotic scars
[49] Fistula [serous exudates]
[50] Fistula [serofibrinous exudates]
[51] Fistula [purulent exudates]
[52] Fistula [haemorrhagic exudates]
[53] Fluid accumulation
[54] Gas accumulation
[55] Abnormal convex shape causing asymmetry
[56] Abnormal concave shape causing asymmetry
[57] Indurations
[58] Other: *[Max. 30characters and no space]*
[59] Not Sure / Not Done

Bones & Hooves

[60] Fracture / Fisures
[61] Thickening
[62] Stress lines [applies to hooves]
[63] White line disorder: Serum tinged [soles only]
[64] White line disorder: Blood tinged [soles only]
[65] Abnormal convex shape causing asymmetry
[66] Abnormal concave shape causing asymmetry
[67] Grown out
[68] Other: ***[Max. 30characters and no space]***
[69] Not Sure / Not Done

Special Organs: Eye Balls

[70] Sunken
[71] Protruding
[72] Blepharospasm [closed eye due to pain]
[73] Internal haemorrhages
[74] Cataract
[75] Thelazia
[76] Other: *[Max. 30characters and no space]*
[77] Not Sure / Not Done

[a] Refers to third eyelid, rectum, vagina, prepuce [penis]…

15. Joints

	Normal	Abnormal	ND					
Shoulder joint	□	□	□	___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____
Elbow Joint	□	□	□	___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____
Carpal joint	□	□	□	___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____
Fetlock joint forelimb	□	□	□	___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____
Hip joint	□	□	□	___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____
Stifle Joint	□	□	□	___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____
Tarsal joint	□	□	□	___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____
Fetlock joint hindlimb	□	□	□	___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____

If abnormal, select lesions from list below. For each lesion you tick, enter 1 = Right; 2 = Left; 3 = Bilateral; ND = Not sure, not done.

[1] Pain	[6] fistula [purulent]	[11] Fracture [Loose]
[2] Swollen	[7] fistula [fibrinopurulent]	[12] Fixed / Rigid
[3] Abscess	[8] fistula [haemorrhagic]	[13] Other: *[Max. 30characters and no space]*
[4] fistula [serous]	[9] In abnormal extension	[ND] Not Sure, not done
[5] fistula [serofibrinous]	[10] In abnormal flexion	

16. Muscular Tone

	Normal	Abnormal	ND			Normal	Abnormal	ND	
Ear [RL]	□	□	□	___[ ]	Anus	□	□	□	___[ ]
Lip [DV]	□	□	□	___[ ]	Tail	□	□	□	___[ ]
Tongue [DV]	□	□	□	___[ ]	Ant body	□	□	□	___[ ]
Neck	□	□	□	___[ ]	Post body	□	□	□	___[ ]
Forelimb [RL]	□	□	□	___[ ]	Whole body	□	□	□	___[ ]
Hindlimb [RL]	□	□	□	___[ ]					

If abnormal, select lesions from list below. For each lesion you tick, enter 1 = Right / dorsal; 2 = Left / ventral; 3 = Bilateral; 4 = Not applicable because the body part is not bilateral.

ND = Not sure, not done.

[1] Reduced tone	[3] Increased tone	[ND] Not sure, not done
[2] No tone	[4] Severe stiffness /Spasm	

17. Faeces

Consistency: Hard□ Normal□ Diarrhoea□ ND□

Type of Diarrhoea: No diarrhoea present [NA] □ Diarrhoea present; enter combined score as explained below □

Use a combined score to define the type of diarrhoea, using the table on the right:

For example, if the diarrhoea is moderate, haemorrhagic and foul-smelling, the code = 243

	First Digit = Severity	Second Digit = Type	Third digit = Odour
1	Mild	Watery	Normal
2	Moderate	Catarrhal	Sour
3	Severe	Mucohaemorrhagic	Foul-smelling
4	Don't Know	Haemorrhagic	Don't Know
5		Other	
6		Don't Know	

Comb Score

18. Tick & Lice

The following section should be completed in all visits. To score the degree of infestation by *R. appendiculatus*, you will only need to observe the ears of the calf. For all other tick groups [as well as lice] you will need to explore ½ the body surface, the perianal area and the tail brush. Tick 'ND' if you could not assess the level of infestation or you are unsure.

	None	Present	Severe	ND
Adult *Rhipicephalus appendiculatus*	□	□	□	□
Adult Amblyomma Ticks	□	□	□	□
Adult Boophilus Ticks	□	□	□	□
Adult Hyalomma Ticks	□	□	□	□
Other Adult Tick Species/Genus	□	□	□	□
Lice	□	□	□	□

18. Tick & Lice [Continued]

If you have detected a severe infestation, you will need to collect 3-6 none engorged ticks [preferably males] for the corresponding tick category. For example, if you detected severe amblyomma infestation, you will collect 3-6 ticks for each amblyomma species responsible for the heavy infestation, and you will place all amblyomma species in the same tube. You would proceed the same way for ticks from the boophilus group, the hyalomma group, etc. For each tick category, tick 'NA' if sample collection is not required [i.e. no severe infestation]. For each tick category, tick 'ND' if sample collection was applicable but not possible. Choose 'TKS' labels to barcode the samples.

Sample Collection

Sample ID	Number of Ticks per Sample	ND	NA
R. appendiculatus / Ear ticks *3 – 6 non engorged adults of each Amblyomma type. Preferably males.*		☐	☐
Ammblyomma spp. *3 – 6 non engorged adults of each Amblyomma type. Preferably males.*			
Boophilus spp. *3 – 6 non engorged adults of each Boophilus type. Preferably males.*		☐	☐
Hyalomma spp. *3 – 6 non engorged adults of each Hyalomma type. Preferably males.*		☐	☐
Other Tick genus / Species *3 – 6 non engorged adults of each 'Other Ticks' type. Preferably males.*		☐	☐

19. Body Measurement & Weight

Weight should be recorded at 7D / 5-weekly visits other than VRC41 and VRC46 and at yearly visits. Tick 'NA' if this is a weekly visit or a CE visit outside the frame of routine visits.

Girth _____ cm ☐ ND

Weight _____ kg ☐ ND ☐ NA

20. Compulsory Sample Collection

Marginal ear vein smears, Plain blood, EDTA blood and faecal samples are compulsory in all visits [including any clinical episode visit] except for weekly visits with no clinical episodes. Click 'NA' [not applicable] if this is a weekly visit with no clinical episode. Click 'ND' [not done] if sample collection was applicable but not possible. Collection of EDTA-Magic buffer blood is compulsory at the 7D visit [visit number 1], at quarterly visits [visit numbers 21 and 41] at and at the yearly visit [visit number 51]. Blood in heparin for storage purposes is compulsory for visits where a clinical episode is detected. Click 'NA' [not applicable] if this is not a clinical episode. Click 'ND' [not done] if sample collection was applicable but not possible. Skin snips from ventral midline [between umbilicus and udder], blood in heparin for immunology studies and the diagnosis of tuberculosis and blood with RNALater are only compulsory in yearly visits [visit number 51]. Click 'NA' [not applicable] if this is not a yearly visit. Click 'ND' [not done] if sample collection was applicable but not possible. If you require skin snips from a clinical episode, please refer to the sample collection form for clinical episodes and leave this as 'NA'. If this is a clinical episode and you require additional heparinised blood for bacteriology, please refer to the sample collection form for clinical episodes.

Select types from list below and enter Barcode ID. The minimum number of samples required is shown in brackets. You have the option of collecting additional samples:

[a] Marginal vein thin smear [n = 2]:

ID _____ ND☐

Numb _____ NA☐

[a] Plain 10 ml vacutainer [n = 1; 2 if yearly visit]:

ID _____ ND☐

Numb _____ NA☐

EDTA - Magic Buffer 10 ml tube [n = 2]:

ID _____ ND☐

Numb _____ NA☐

[a] Marginal vein thick smear [n = 2]:

ID _____ ND☐

Numb _____ NA☐

[a] EDTA 5 ml vacutainer [n = 2]:

ID _____ ND☐

Numb _____ NA☐

[a] Faecal Sample [n = 2]:

ID _____ ND☐

Numb _____ NA☐

[a] Heparin 10 ml vacutainer FOR STORAGE PURPOSES ONLY [n = 1]

ID _____ ND☐

Numb _____ NA☐

Skin snip ventral midline [n = 1]:

ID _____ ND☐

Numb _____ NA☐

Heparin 10 ml vacutainer for immunology and TBC [n = 2]

ID _____ ND☐

Numb _____ NA☐

EDTA - RNALater 1.8 ml tube [n = 4]:

ID _____ ND☐

Numb _____ NA☐

[a] *Compulsory in clinical episodes*

21. Further Sample collection: Clinical Episodes

1. Have you detected a clinical episode?

☐ Yes ☐ No

2. Are you proceeding with additional sample collection for diagnosis of clinical episode?

☐ Yes ☐ No [Not Applicable] ☐ Applicable but not possible

If yes, move on to the clinical episodes form for Calves after answering the 'Congenital Disorders' question. If no, answer the congenital disorders question and close questionnaire.

22. Congenital Disorders

1. Over the course of the clinical examination, have you observed any congenital disorder?

☐ Yes ☐ No

If yes, move on to the Congenital Disorders form for Calves. If no, stop here.

23. Pictures

It is compulsory that you take AT LEAST one picture from each lesion for later validation of the current classification of lesions. You must save your pictures electronically, but a paper copy of the pictures is also required. It is compulsory that you attach a copy of your pictures here:

PTC ID [Attach Label]: _____

Description (Questionnaire Section, Body Part, Lesion Code, etc.):

_____

_____

_____

_____

_____

PTC ID [Attach Label]: _____

Description (Questionnaire Section, Body Part, Lesion Code, etc.):

_____

_____

_____

_____

_____

PTC ID [Attach Label]: _____

Description (Questionnaire Section, Body Part, Lesion Code, etc.):

_____

_____

_____

_____

_____

PTC ID [Attach Label]: _____

Description (Questionnaire Section, Body Part, Lesion Code, etc.):

_____

_____

_____

_____

_____

PTC ID [Attach Label]: _____

Description (Questionnaire Section, Body Part, Lesion Code, etc.):

_____

_____

_____

_____

_____

PTC ID [Attach Label]: _____

Description (Questionnaire Section, Body Part, Lesion Code, etc.):

_____

_____

_____

_____

_____

Appendix F

Post-mortem questionnaire

This questionnaire was delivered when a calf died. Some sections relating to the farm were delivered to the farmer on collection of the carcass. Later sections are completed during the post-mortem examination.

1. Visit Details

Calf ID **AHA / Vet ID**

Visit ID **Date:** _____ **Time:** _____

Visit Type: ■Post-Mortem Visit

2. Inter-Visit History: Weaning

1. Was the calf still suckling milk from the dam? □ Yes □ No □ Unknown

3. Death History

1. Date of Death: [ ] Unknown [ ]

2. Time of Death: [ ] Unknown [ ]

3. Type of death:

□ Known □ Unknown If Known, select from List below:

□ Ante-Natal [Abortion] □ Neonatal [Within 48 h after Birth] □ Euthanasia

□ At Calving □ Post - Neonatal

4. History of Death

□ Known □ Unknown If Known, select from List below:

□ Trauma / Accident (includes snake bites) □ Euthanasia

□ Congenital Disorder that is externally apparent □ Neither of these

5. Are you proceeding with a post-mortem examination?

□ Yes □ No □ NA [Not Applicable] □ Applicable but not possible

Click on 'NA' [Not Applicable] if you selected 'Trauma / Accident' or 'congenital disorder that is externally apparent ' as the reasons for death in section 4. In such cases you will terminate the questionnaire here. If you have observed a congenital defect that is externally apparent, close this questionnaire after answering question 5 and move on to the congenital disorders form for calves.

4. Inter-Visit History: Animal Movements, Mortality & Animal Bites

1. Write down the number of NEW animals in the herd since [In] and the numbers of animals that have left the herd [Out] since the last visit to the farm with inter-visit history. The latter includes losses due to deaths. Also record the number of animals currently in the herd [Current] and the animals that have died during the inter-visit period.

Please note that you should also consider the dam and the calf as part of the herd. So you need to count these animals in the corresponding categories and when doing the total counts. Follow instructions as described in clinical examination form for calves.

	IN			OUT			CURRENT			DEAD			REASONS FOR DEATH
	N	ND	> 0	N	ND	>0	NA	N	ND	> 0	N	ND	>0	NA	____ [ ] ____ [ ] ____ [ ] ____ [ ]
CBH-M		□	□		□	□	□		□	□		□	□	□	____ [ ] ____ [ ] ____ [ ] ____ [ ]
CBH-F		□	□		□	□	□		□	□		□	□	□	____ [ ] ____ [ ] ____ [ ] ____ [ ]
CNBH-M		□	□		□	□	□		□	□		□	□	□	____ [ ] ____ [ ] ____ [ ] ____ [ ]
CNBH-F		□	□		□	□	□		□	□		□	□	□	____ [ ] ____ [ ] ____ [ ] ____ [ ]
W-M		□	□		□	□	□		□	□		□	□	□	____ [ ] ____ [ ] ____ [ ] ____ [ ]
W-F		□	□		□	□	□		□	□		□	□	□	____ [ ] ____ [ ] ____ [ ] ____ [ ]
A-M		□	□		□	□	□		□	□		□	□	□	____ [ ] ____ [ ] ____ [ ] ____ [ ]
A-F		□	□		□	□	□		□	□		□	□	□	____ [ ] ____ [ ] ____ [ ] ____ [ ]
TOTAL		□	□		□	□	□		□	□		□	□	□	____ [ ] ____ [ ] ____ [ ] ____ [ ]

If there is a history of deaths, enter the reasons for death as follows: **1** = Trauma / Accident; **2** = Animal bite; **3** = Born dead / abortion; **4** = Dead during calving; **5** = Dead 48 h after birth for reasons other that 1 or 2; **6** = Dead > 48 h after birth for reasons other that 1 or 2; **ND** = Not done, don't know. For each reason, you must specify the number of affected animals [either a **number** or '**ND**' if you don't know how many animals died due to the specified reason].

2. Is there a history of animal bites between this visit and the previous [7D/monthly] visit?
If the dam is no longer in the herd, click 'NA'. If there are no other animals in the herd, click 'NA'.

	Yes	No	Unknown	NA
Calf	□	□	□	
Dam	□	□	□	□
Other animals in herd	□	□	□	□

If yes, tick one or more options from list below.

□ Dog bite	□ Snake bite	□ Other_____	□ Don't know
□ Dog bite	□ Snake bite	□ Other_____	□ Don't know
□ Dog bite	□ Snake bite	□ Other_____	□ Don't know

5. Inter-Visit History: Veterinary Interventions

1. Is there a history of veterinary interventions between this visit and the last visit with inter-visit history?

For the case of the dam, and for the case of other animals in the herd, you will tick 'NA' [not applicable] if the dam is no longer in the farm [i.e if sold, dead, etc.] or if there are no other animals in the herd.

If yes, select the type/s of treatment from below. In the case of 'other animals in the herd', for each treatment you tick, enter the number of treated animals. If you are not sure about the exact number of treated animals, enter one of the following scores: **A** = Up to 10% of the animals; **B** = Approximately > 10% but < 50% of the animals. **C =** Approximately 50% of the animals [~50%]; **D** = Most animals [> 50% herd]; **E** = All animals in herd; **ND** = Don't know. For the case of other animals in the herd, if you enter the number of treated animals, make sure that you have entered the 'current number of animals in the herd' in the previous section. For the case of Insecticides / acaricides, for each product you enter, you must enter a code to indicate the type of application as follows: **1** = spraying of legs only; **2** = spraying of whole body; **3** = Dipping; **4** = Pour on; **ND** = Don't know.

Calf	**Dam**	**Other animals in the herd**
□**Yes** □**No** □**ND [Not Sure/Not done]**	□**Yes** □**No** □**ND [Not Sure/Not done]** □**NA**	□**Yes** □**No** □**ND [Not Sure/Not done]** □**NA**
If yes, select from list below:	*If yes, select from list below:*	*If yes, select from list below:*
Antihelminthics □**Yes**	Antihelminthics □**Yes**	Antihelminthics □**Yes**
□ Albendazole	□ Albendazole	□ Albendazole N ___
□ Ivermectin	□ Ivermectin	□ Ivermectin N ___
□ Other_____	□ Other_____	□ Other_____ N ___
□ Not Sure, don't know	□ Not Sure, don't know	□ Not Sure, don't know N ___
Antibiotics □**Yes**	Antibiotics □**Yes**	Antibiotics □**Yes**
□ LA oxytetracycline	□ LA oxytetracycline	□ LA oxytetracycline N ___
□ SA oxytetracycline	□ SA oxytetracycline	□ SA oxytetracycline N ___
□ Other_____	□ Other_____	□ Other_____ N ___
□ Not Sure, don't know	□ Not Sure, don't know	□ Not Sure, don't know N ___
Trypanocidals □**Yes**	Trypanocidals □**Yes**	Trypanocidals □**Yes**
□ Diminazene	□ Diminazene	□ Diminazene N ___
□ Isomethamidium	□ Isomethamidium	□ Isomethamidium N ___
□ Homidium salts	□ Homidium salts	□ Homidium salts N ___
□ Other_____	□ Other_____	□ Other_____ N ___
□ Not Sure, don't know	□ Not Sure, don't know	□ Not Sure, don't know N ___
Antiprotozoals □**Yes**	Antiprotozoals □**Yes**	Antiprotozoals □**Yes**
□ Imidocarb	□ Imidocarb	□ Imidocarb N ___
□ Parvaquone	□ Parvaquone	□ Parvaquone N ___
□ Buparvaquone	□ Buparvaquone	□ Buparvaquone N ___
□ Other_____	□ Other_____	□ Other_____ N ___
□ Not Sure, don't know	□ Not Sure, don't know	□ Not Sure, don't know N ___
Insecticides/Acaricides □**Yes**	Insecticides/Acaricides □**Yes**	Insecticides/Acaricides □**Yes**
□ Deltamethrone A [ ]	□ Deltamethrone A [ ]	□ Deltamethrone A [ ] N ___
□ Other_____ A [ ]	□ Other_____ A [ ]	□ Other_____ A [ ] N ___
□ Not Sure, don't know A [ ]	□ Not Sure, don't know A [ ]	□ Not Sure, don't know A [ ] N ___
Traditional □**Yes**	Traditional □**Yes**	Traditional □**Yes**
□ Paraffin	□ Paraffin	□ Paraffin N ___
□ Tick grease	□ Tick grease	□ Tick grease N ___
□ Manual removal of ticks	□ Manual removal of ticks	□ Manual removal of ticks N ___
□ Burning of lymph nodes	□ Burning of lymph nodes	□ Burning of lymph nodes N ___
□ Other_____	□ Other_____	□ Other_____ N ___
□ Not Sure, don't know	□ Not Sure, don't know	□ Not Sure, don't know N ___
Other □**Yes**	Other □**Yes**	Other □**Yes**
□ Topical Antiseptic	□ Topical Antiseptic	□ Topical Antiseptic N ___
□ Vitamin supplementation	□ Vitamin supplementation	□ Vitamin supplementation N ___
□ Other_____	□ Other_____	□ Other_____ N ___
□ Not Sure, don't know	□ Not Sure, don't know	□ Not Sure, don't know N ___
Vaccines □**Yes**	Vaccines □**Yes**	Vaccines □**Yes**
□ Muguga cocktail	□ Muguga cocktail	□ Muguga cocktail N ___
□ CBPP	□ CBPP	□ CBPP N ___
□ FMD	□ FMD	□ FMD N ___
□ LSD	□ LSD	□ LSD N ___
□ BQ	□ BQ	□ BQ N ___
□ Anthrax	□ Anthrax	□ Anthrax N ___
□ Brucella	□ Brucella	□ Brucella N ___
□ Other_____	□ Other_____	□ Other_____ N ___
□ Not Sure, don't know	□ Not Sure, don't know	□ Not Sure, don't know N ___
Unknown Category □**Yes**	Unknown Category □**Yes**	Unknown Category □**Yes**
□ Other_____	□ Other_____	□ Other_____ N ___
□ Not Sure, don't know	□ Not Sure, don't know	□ Not Sure, don't know N ___

6. Inter-visit history & Inspection at Rest: Herd Health

For each disorder present in the herd, you must enter a combined score. The first digit indicates **who has seen the lesions**: Enter **1** if you observe the disorder and enter **2** if you can't see the disorder, but the farmer has observed the disorder during the inter-visits time. The second digit in the score indicates **whether the dam is affected**: **1** = yes / **2** = no / **3** = don't know / **4** = The dam is no longer in the herd. The third digit in the score indicates the **extent of the problem at the herd level** [excluding the dam]: **1** = No affected animals; **2** = Up to 10% of the animals; **3** = Approximately > 10% but < 50% of the animals. **4** = Approximately 50% of the animals [~50%]; **5** = Most animals [> 50% herd]; **6** = All animals in herd; **7** = Don't know; **8** = There are no other animals in the herd.

e.g. If the farmer has observed a disorder for the case of the dam and all other animals in herd, which you have not seen during the visit, the code is: 216.

Feeding/Drinking

□ **Normal** □ **Abnormal** □ **Unknown**

If abnormal select from below:

□ Unable to swallow food [ ]
□ Food apprehension [ ]
□ Anorexia [ ]
□ Decreased appetite [ ]
□ Increased water intake [ ]
□ Decreased water intake [ ]
□ Other_____[ ]
□ Not sure / Not done [ ]

Mouth

□ **Normal** □ **Abnormal** □ **Unknown**

If abnormal select from below:

□ Hyperaemia [ ]
□ Haemorrhages [ ]
□ Blisters [Vesicles / Pustules] [ ]
□ Ulcers/erosions [ ]
□ Scabs / Scars [ ]
□ Other_____[ ]
□ Not sure / Not done [ ]

Feet

□ **Normal** □ **Abnormal** □ **Unknown**

If abnormal select from below:

□ Hyperaemia [ ]
□ Haemorrhages [ ]
□ Blisters [Vesicles / Pustules] [ ]
□ Ulcers/erosions [ ]
□ Scabs / Scars [ ]
□ Other_____[ ]
□ Not sure / Not done [ ]

Skin/Coat

□ **Normal** □ **Abnormal** □ **Unknown**

If abnormal select from below:

□ Generalised alopecia [ ]
□ Nodular lesions [ ]
□ Generalised sloughing [ ]
□ Excessive Sweating [ ]
□ Ulcers / Erosions [ ]
□ Scars / Scabs [ ]
□ Other_____[ ]
□ Not sure / Not done [ ]

Eyes

□ **Normal** □ **Abnormal** □ **Unknown**

If abnormal select from below:

□ Ocular Discharge [ ]
□ Corneal Opacity [ ]
□ Blindness [not nervous condition] [ ]
□ Other_____[ ]
□ Not sure / Not done [ ]

Posture

□ **Normal** □ **Abnormal** □ **Unknown**

If abnormal select from below:

□ Arched back [ ]
□ Recumbency [ ]
□ Extended head and neck [ ]
□ Star-gazing [ ]
□ Wide-based stance [ ]
□ Dog-sitting [ ]
□ Lateral positioning of head [ ]
□ Other_____[ ]
□ Not sure / Not done [ ]

Gait

□ **Normal** □ **Abnormal** □ **Unknown**

If abnormal select from below:

□ Lameness [ ]
□ Stiffness [ ]
□ Limping [ ]
□ Swaying hind quarter [ ]
□ Other_____[ ]
□ Not sure / Not done [ ]

Swelling

□ **Normal** □ **Abnormal** □ **Unknown**

If abnormal select from below:

□ Large muscle groups [ ]
□ Joints [ ]
□ Lymph nodes [ ]
□ Ventral thorax [ ]
□ Ventral abdomen [ ]
□ Other_____[ ]
□ Not sure / Not done [ ]

Nervous / Behaviour Change

□ **Normal** □ **Abnormal** □ **Unknown**

If abnormal select from below:

□ Nystagmus [ ]
□ Blindness [nervous condition] [ ]
□ Muscular tremors / muscle twitching [ ]
□ Convulsions [ ]
□ Incoordination / ataxia [ ]
□ High stepping gait [ ]
□ Circling [ ]
□ General weakness [ ]
□ Reduced sensitivity [ ]
□ Paralysis [ ]
□ Hypersensitivity [ ]
□ Restlessness [ ]
□ Lethargy [ ]
□ Aggression [ ]
□ Excessive licking [ ]

Nervous / Behaviour Change

□ Excessive chewing [ ]
□ Excessive salivation/drooling [ ]
□ Excessive bellowing [ ]
□ Other_____[ ]
□ Not sure / Not done [ ]

Respiratory

□ **Normal** □ **Abnormal** □ **Unknown**

If abnormal select from below:

□ Non-foamy nasal discharge [ ]
□ Foamy nasal discharge [ ]
□ Cough [ ]
□ Costo-abdominal respiration [ ]
□ Shallow / rapid breathing [ ]
□ Deep / laboured breathing [ ]
□ Other_____[ ]
□ Not sure / Not done [ ]

Gastrointestinal

□ **Normal** □ **Abnormal** □ **Unknown**

If abnormal select from below:

□ Tenesmus [straining] [ ]
□ Constipation [ ]
□ Hard faeces: Not bloody [ ]
□ Hard faeces: Bloody [ ]
□ Soiling [ ]
□ Diarrhoea: Not bloody [ ]
□ Diarrhoea: Bloody [ ]
□ Regurgitation / Vomiting [ ]
□ Other_____[ ]
□ Not sure / Not done [ ]

Urinary

□ **Normal** □ **Abnormal** □ **Unknown**

If abnormal select from below:

□ Straining [ ]
□ Excessive urination [ ]
□ Water coloured urine [ ]
□ Reddish-tinged urine [ ]
□ Brownish-tinged urine [ ]
□ Increased consistency of urine [>>density] [ ]
□ Other_____[ ]
□ Not sure / Not done [ ]

Ill Thrift

□ **Normal** □ **Abnormal** □ **Unknown**

If abnormal select from below:

□ Weight loss / loss of condition [ ]
□ Cachexy / extreme thinness [ ]
□ Pale mucous membranes [ ]
□ Icterus [ ]
□ Cyanosis [ ]
□ Rough/staring coat. Not bright/shiny [ ]
□ Weakness [not nervous condition] [ ]
□ Other_____[ ]
□ Not sure / Not done [ ]

7. Inter-visit history & Inspection at Rest: Calf Health

For each disorder you tick, enter **1** if you have observed the disorder when inspecting the carcass and enter **2** if you have not observed the disorder, but the farmer has observed such disorder before the animal died.

Feeding/Drinking

□ **Normal** □ **Abnormal** □ **Unknown**

If abnormal select from below:

□ Unable to swallow food [ ]
□ Food apprehension [ ]
□ Anorexia [ ]
□ Decreased appetite [ ]
□ Increased water intake [ ]
□ Decreased water intake [ ]
□ Other_____[ ]
□ Not sure / Not done [ ]

Mouth

□ **Normal** □ **Abnormal** □ **Unknown**

If abnormal select from below:

□ Hyperaemia [ ]
□ Haemorrhages [ ]
□ Blisters [Vesicles / Pustules] [ ]
□ Ulcers/erosions [ ]
□ Scabs / Scars [ ]
□ Other_____[ ]
□ Not sure / Not done [ ]

Feet

□ **Normal** □ **Abnormal** □ **Unknown**

If abnormal select from below:

□ Hyperaemia [ ]
□ Haemorrhages [ ]
□ Blisters [Vesicles / Pustules] [ ]
□ Ulcers/erosions [ ]
□ Scabs / Scars [ ]
□ Other_____[ ]
□ Not sure / Not done [ ]

Skin/Coat

□ **Normal** □ **Abnormal** □ **Unknown**

If abnormal select from below:

□ Generalised alopecia [ ]
□ Nodular lesions [ ]
□ Generalised sloughing [ ]
□ Excessive Sweating [ ]
□ Ulcers / Erosions [ ]
□ Scars / Scabs [ ]
□ Other_____[ ]
□ Not sure / Not done [ ]

Eyes

□ **Normal** □ **Abnormal** □ **Unknown**

If abnormal select from below:

□ Ocular Discharge [ ]
□ Corneal Opacity [ ]
□ Blindness [not nervous condition] [ ]
□ Other_____[ ]
□ Not sure / Not done [ ]

Posture

□ **Normal** □ **Abnormal** □ **Unknown**

If abnormal select from below:

□ Arched back [ ]
□ Recumbency [ ]
□ Extended head and neck [ ]
□ Star-gazing [ ]
□ Wide-based stance [ ]
□ Dog-sitting [ ]
□ Lateral positioning of head [ ]
□ Other_____[ ]
□ Not sure / Not done [ ]

Gait

□ **Normal** □ **Abnormal** □ **Unknown**

If abnormal select from below:

□ Lameness [ ]
□ Stiffness [ ]
□ Limping [ ]
□ Swaying hind quarter [ ]
□ Other_____[ ]
□ Not sure / Not done [ ]

Swelling

□ **Normal** □ **Abnormal** □ **Unknown**

If abnormal select from below:

□ Large muscle groups [ ]
□ Joints [ ]
□ Lymph nodes [ ]
□ Ventral thorax [ ]
□ Ventral abdomen [ ]
□ Other_____[ ]
□ Not sure / Not done [ ]

Nervous / Behaviour Change

□ **Normal** □ **Abnormal** □ **Unknown**

If abnormal select from below:

□ Nystagmus [ ]
□ Blindness [nervous condition] [ ]
□ Muscular tremors / muscle twitching [ ]
□ Convulsions [ ]
□ Incoordination / ataxia [ ]
□ High stepping gait [ ]
□ Circling [ ]
□ General weakness [ ]
□ Reduced sensitivity [ ]
□ Paralysis [ ]
□ Hypersensitivity [ ]
□ Restlessness [ ]
□ Lethargy [ ]
□ Aggression [ ]
□ Excessive licking [ ]

Nervous / Behaviour Change

□ Excessive chewing [ ]
□ Excessive salivation/drooling [ ]
□ Excessive bellowing [ ]
□ Other_____[ ]
□ Not sure / Not done [ ]

Respiratory

□ **Normal** □ **Abnormal** □ **Unknown**

If abnormal select from below:

□ Non-foamy nasal discharge [ ]
□ Foamy nasal discharge [ ]
□ Cough [ ]
□ Costo-abdominal respiration [ ]
□ Shallow / rapid breathing [ ]
□ Deep / laboured breathing [ ]
□ Other_____[ ]
□ Not sure / Not done [ ]

Gastrointestinal

□ **Normal** □ **Abnormal** □ **Unknown**

If abnormal select from below:

□ Tenesmus [straining] [ ]
□ Constipation [ ]
□ Hard faeces: Not bloody [ ]
□ Hard faeces: Bloody [ ]
□ Soiling [ ]
□ Diarrhoea: Not bloody [ ]
□ Diarrhoea: Bloody [ ]
□ Regurgitation / Vomiting [ ]
□ Other_____[ ]
□ Not sure / Not done [ ]

Urinary

□ **Normal** □ **Abnormal** □ **Unknown**

If abnormal select from below:

□ Straining [ ]
□ Excessive urination [ ]
□ Water coloured urine [ ]
□ Reddish-tinged urine [ ]
□ Brownish-tinged urine [ ]
□ Increased consistency of urine [>>density] [ ]
□ Other_____[ ]
□ Not sure / Not done [ ]

Ill Thrift

□ **Normal** □ **Abnormal** □ **Unknown**

If abnormal select from below:

□ Weight loss / loss of condition [ ]
□ Cachexy / extreme thinness [ ]
□ Pale mucous membranes [ ]
□ Icterus [ ]
□ Cyanosis [ ]
□ Rough/staring coat. Not bright/shiny [ ]
□ Weakness [not nervous condition] [ ]
□ Other_____[ ]
□ Not sure / Not done [ ]

8. Lymph Nodes

	Normal	Abnormal	ND					
Parotid Lymph node	☐	☐	☐	___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____
Supra-scapular lymph node	☐	☐	☐	___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____
Pre-crural lymph node	☐	☐	☐	___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____

If abnormal select lesions from list below. For each lesion you tick, enter 1 = Right; 2 = Left; 3 = Bilateral; ND = Not sure / not done:

[1] Hyperplasic	[2] Atrophied	[3] Fistula	[4] Abscess	[5] Other: *[Max. 30characters no space]*	[ND] Not sure / not done

9. Calliper measures

Supra-scapular	**Right horizontal**		cm	☐ ND
	Left horizontal		cm	☐ ND
Pre-Crural	**Right horizontal**		cm	☐ ND
	Left horizontal		cm	☐ ND

10. Natural Openings: Discharge and Exudates

	None	Mild	Profuse	ND	NA					
Ocular[RL]	☐	☐	☐	☐		___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____
Nasal[RL]	☐	☐	☐	☐		___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____
Ear[RL]	☐	☐	☐	☐		___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____
Umbilicus	☐	☐	☐	☐		___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____
Udder	☐	☐	☐	☐	☐	___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____
Vaginal	☐	☐	☐	☐	☐	___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____
Prepucial	☐	☐	☐	☐	☐	___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____

If abnormal select type of discharge from list below [code]. For each lesion you select, enter 1 = Right; 2 = Left; 3 = Bilateral; 4 = Not applicable because the body part is not bilateral; ND = Not sure / not done:

[1] Serous discharge	[5] Purulent discharge	[9] Free blood
[2] Serofibrinous discharge	[6] Blood-tinged discharge	[10] Other: *[Max. 30characters and no space]*
[3] Mucus discharge	[7] Clear foam	[ND] Not sure, not done
[4] Mucopurulent discharge	[8] Blood-tinged foamy	

Normally, discharge types vary across natural openings as follows: Serofibrinous [applies to nasal, ocular]; Mucus or mucopurulent [applies to ocular / nasal/ vaginal]; Purulent discharge [applies to ear/nasal/navel/udder/vagina]; foamy [applies to nasal].

11. Joints

	Normal	Abnormal	ND					
Shoulder joint	□	□	□	___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____
Elbow Joint	□	□	□	___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____
Carpal joint	□	□	□	___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____
Fetlock joint forelimb	□	□	□	___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____
Hip joint	□	□	□	___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____
Stifle Joint	□	□	□	___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____
Tarsal joint	□	□	□	___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____
Fetlock joint hindlimb	□	□	□	___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____

If abnormal, select lesions from list below. For each lesion you tick, enter 1 = Right; 2 = Left; 3 = Bilateral; ND = Not sure, not done.

[1] Pain	[6] fistula [purulent]	[11] Fracture [Loose]
[2] Swollen	[7] fistula [fibrinopurulent]	[12] Fixed / Rigid
[3] Abscess	[8] fistula [haemorrhagic]	[13] Other: *[Max. 30characters and no space]*
[4] fistula [serous]	[9] In abnormal extension	[ND] Not Sure, not done
[5] fistula [serofibrinous]	[10] In abnormal flexion	

12. Weight of Carcass (Kg)

Use the hanging scale if the calf died up to VRC31. Use the weight beams if the calf died after VRC31.

Weight (Kg): Scale Used: □ Weight Beam □ Hanging Scale

13. Tick & Lice

To score the degree of infestation by *R. appendiculatus*, you will only need to observe the ears of the calf. For all other tick groups [as well as lice] you will need to explore ½ the body surface, the perianal area and the tail brush. Tick 'ND' if you could not assess the level of infestation or you are unsure.

	None	Present	Severe	ND
Adult *Rhipicephalus appendiculatus*	☐	☐	☐	☐
Adult Amblyomma Ticks	☐	☐	☐	☐
Adult Boophilus Ticks	☐	☐	☐	☐
Adult Hyalomma Ticks	☐	☐	☐	☐
Other Adult Tick Species/Genus	☐	☐	☐	☐
Lice	☐	☐	☐	☐

13. Tick & Lice [Continued]

If you have detected a severe infestation, you will need to collect 3-6 none engorged ticks [preferably males] for the corresponding tick category. For example, if you detected severe amblyomma infestation, you will collect 3-6 ticks for each amblyomma species responsible for the heavy infestation, and you will place all amblyomma species in the same tube. You would proceed the same way for ticks from the boophilus group, the hyalomma group, etc. For each tick category, tick 'NA' if sample collection is not required [i.e. no severe infestation]. For each tick category, tick 'ND' if sample collection was applicable but not possible. Chose 'TKS' labels to barcode the samples.

Sample Collection

Sample ID	**Number of Ticks per Sample**	**ND**	**NA**
R. appendiculatus / Ear ticks *3 – 6 non engorged adults of each Amblyomma type. Preferably males.*		☐	☐
Ammblyomma spp. *3 – 6 non engorged adults of each Amblyomma type. Preferably males.*			
Boophilus spp. *3 – 6 non engorged adults of each Boophilus type. Preferably males.*		☐	☐
Hyalomma spp. *3 – 6 non engorged adults of each Hyalomma type. Preferably males.*		☐	☐
Other Tick genus / Species *3 – 6 non engorged adults of each 'Other Ticks' type. Preferably males.*		☐	☐

14. Skin, Mucous Membranes, Muscles, Abdomen, Bones, Hooves and Special Organs

	Normal	Abnormal	ND						
Face [RL]	□	□	□	___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] :_____	___ [ ] :_____
Head [not face; RL]	□	□	□	___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] :_____	___ [ ] :_____
Ear [RL]	□	□	□	___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] :_____	___ [ ] :_____
Eye ball [RL]	□	□	□	___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] :_____	___ [ ] :_____
Conjunctiva [RL]	□	□	□	___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] :_____	___ [ ] :_____
Cornea [RL]	□	□	□	___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] :_____	___ [ ] :_____
Sclera [RL]	□	□	□	___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] :_____	___ [ ] :_____
Eyelid [RL]	□	□	□	___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] :_____	___ [ ] :_____
Third eyelid [RL]	□	□	□	___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] :_____	___ [ ] :_____
Muzzle	□	□	□	___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] :_____	___ [ ] :_____
Nostrils [RL]	□	□	□	___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] :_____	___ [ ] :_____
Lips [DV]	□	□	□	___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] :_____	___ [ ] :_____
Gums [DV]	□	□	□	___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] :_____	___ [ ] :_____
Palate	□	□	□	___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] :_____	___ [ ] :_____
Tongue [DV]	□	□	□	___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] :_____	___ [ ] :_____
Mouth floor [interior]	□	□	□	___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] :_____	___ [ ] :_____
Cheeks [interior; RL]	□	□	□	___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] :_____	___ [ ] :_____
Inter-mandibular sp. [ext.]	□	□	□	___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] :_____	___ [ ] :_____
Neck [DV]	□	□	□	___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] :_____	___ [ ] :_____
Lateral neck [RL]	□	□	□	___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] :_____	___ [ ] :_____
Forelimb [RL]	□	□	□	___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] :_____	___ [ ] :_____
				___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] :_____	___ [ ] :_____
				___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] :_____	___ [ ] :_____
				___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] :_____	___ [ ] :_____
				___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] :_____	___ [ ] :_____

If abnormal, select from list below. For each body part you may select as many skin lesions / Muscular lesions / Bones, Hooves lesions / Special Organ lesions as you see. For each lesion that you enter you must enter a combined score. The first digit on the score indicates the **location of the lesion**: 1 = Right or dorsal / 2 = Left or ventral; 3 = Bilateral [Both RL or DV]; 4 = Not applicable because the body part is not bilateral; 5 = Not sure, not done. The second digit in the score indicates the **extent of the lesion**: 1 = Focal ; 2 = Multi-focal; 3 = Diffuse; 4 = Mixture of 1 & 2 ; 5 = Mixture of 1 & 3; 6 = Mixture of 2 & 3; 7 = Not sure, not done. e.g. If you have multi-focal abscesses in the skin of the right forelimb, and a single abscess in the skin of the left forelimb, you would click 'Skin', then 'Abscesses' and you would enter code = 34.

Skin & Mucous Membranes	[27] Fibrotic scars	[53] Fluid accumulation
[1] Alopecia	[28] Crusts	[54] Gas accumulation
[2] Sloughing	[29] Necrosis	[55] Abnormal convex shape causing asymmetry
[3]	[30] Gangrene [not in mucous membranes]	[56] Abnormal concave shape causing asymmetry
[4] Swelling/oedema	[31] Foul-odour/Malodour	[57] Indurations
[5] Dry/scaly appearance [Hyperkeratosis]	[32] Abscesses	[58] Other: *[Max. 30characters and no space]*
[6] Pallor [Pale]	[33] Cracks	[59] Not Sure / Not Done
[7] Hyperaemia/congestion	[34] Loss of papillae [dorsal tongue only]	Bones & Hooves
[8] Icterus [yellowish]	[35] Foreign bodies	[60] Fracture / Fisures
[9] Cyanosis [bluish]	[36] Opacity [Cornea]	[61] Thickening
[10] Haemorrhages	[37] Perforation	[62] Stress lines [applies to hooves]
[11] Non-exudative nodules/granules	[38] Prolapse [a]	[63] White line disorder: Serum tinged [soles only]
[12] Exudative nodules/granules	[39] Other: *[Max. 30characters no space]*	[64] White line disorder: Blood tinged [soles only]
[13] Non-exudative raised clumps of hair	[40] Not Sure / Not Done	[65] Abnormal convex shape causing asymmetry
[14] Exudative raised clumps of hair	Muscles & Abdomen	[66] Abnormal concave shape causing asymmetry
[15] 'Bleeding spots'	*[41]*	[67] Grown out
[16] 'Breathing holes' [warbles]	[42] Swelling/oedema/enlargement	[68] Other: ***[Max. 30characters and no space]***
[17] Myasis	[43] Atrophy [smaller than normal]	[69] Not Sure / Not Done
[18] Other macroscopic ectoparasites	[44] Gangrene	Special Organs: Eye Balls
[19] Blisters [vesicles/ pustules/papules]	[45] Nodules	[70] Sunken
[20] Warts	[46] Abscesses	[71] Protruding
[21] Non-exudative ulcers/erosions	[47] Scabs	*[72]*
[22] Ulcers/erosions [serous exudates]	[48] Fibrotic scars	[73] Internal haemorrhages
[23] Ulcers/erosions [serofibrinous exudates]	[49] Fistula [serous exudates]	[74] Cataract
[24] Ulcers/erosions [purulent exudates]	[50] Fistula [serofibrinous exudates]	[75] Thelazia
[25] Ulcers/erosions [haemorrhagic exudates]	[51] Fistula [purulent exudates]	[76] Other: *[Max. 30characters and no space]*
[26] Scabs	[52] Fistula [haemorrhagic exudates]	[77] Not Sure / Not Done

[a] Refers to third eyelid, rectum, vagina, prepuce [penis]…

14. Skin, Mucous Membranes, Muscles, Abdomen, Bones, Hooves and Special Organs [Continued]

	Normal	Abnormal	ND	NA						
Cor band forelimb [RL]	□	□	□		___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] : _____	___ [ ] : _____
Hoof forelimb [RL]	□	□	□		___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] : _____	___ [ ] : _____
Inter-digital forelimb [RL]	□	□	□		___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] : _____	___ [ ] : _____
Sole forelimb [RL]	□	□	□		___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] : _____	___ [ ] : _____
Thorax [DV]	□	□	□		___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] : _____	___ [ ] : _____
Lateral thorax [RL]	□	□	□		___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] : _____	___ [ ] : _____
Abdomen [DV]	□	□	□		___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] : _____	___ [ ] : _____
Umbilicus	□	□	□		___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] : _____	___ [ ] : _____
Udder	□	□	□	□	___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] : _____	___ [ ] : _____
Testis [RL]	□	□	□	□	___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] : _____	___ [ ] : _____
Penis	□	□	□	□	___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] : _____	___ [ ] : _____
Lateral abdomen [RL]	□	□	□		___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] : _____	___ [ ] : _____
Hindlimb [RL]	□	□	□		___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] : _____	___ [ ] : _____
Cor band hindlimb [RL]	□	□	□		___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] : _____	___ [ ] : _____
Hoof hindlimb [RL]	□	□	□		___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] : _____	___ [ ] : _____
Inter-digital hindlimb [RL]	□	□	□		___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] : _____	___ [ ] : _____
Sole hindlimb [RL]	□	□	□		___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] : _____	___ [ ] : _____
Perianal area	□	□	□		___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] : _____	___ [ ] : _____
Anus	□	□	□		___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] : _____	___ [ ] : _____
Vulva	□	□	□	□	___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] : _____	___ [ ] : _____
Tail	□	□	□		___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] : _____	___ [ ] : _____
	□	□	□		___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] : _____	___ [ ] : _____
					___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] : _____	___ [ ] : _____
					___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] : _____	___ [ ] : _____
					___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] : _____	___ [ ] : _____

If abnormal, select from list below. For each body part you may select as many skin lesions / Muscular lesions / Bones, Hooves lesions / Special Organ lesions as you see. For each lesion that you enter you must enter a combined score. The first digit on the score indicates the **location of the lesion**: 1 = Right or dorsal / 2 = Left or ventral; 3 = Bilateral [Both RL or DV]; 4 = Not applicable because the body part is not bilateral; 5 = Not sure, not done. The second digit in the score indicates the **extent of the lesion**: 1 = Focal ; 2 = Multi-focal; 3 = Diffuse; 4 = Mixture of 1 & 2 ; 5 = Mixture of 1 & 3; 6 = Mixture of 2 & 3; 7 = Not sure, not done. e.g. If you have multi-focal abscesses in the skin of the right forelimb, and a single abscess in the skin of the left forelimb, you would click 'Skin', then 'Abscesses' and you would enter code = 34.

Skin & Mucous Membranes	[27] Fibrotic scars	[53] Fluid accumulation
[1] Alopecia	[28] Crusts	[54] Gas accumulation
[2] Sloughing	[29] Necrosis	[55] Abnormal convex shape causing asymmetry
[3]	[30] Gangrene [not in mucous membranes]	[56] Abnormal concave shape causing asymmetry
[4] Swelling/oedema	[31] Foul-odour/Malodour	[57] Indurations
[5] Dry/scaly appearance [Hyperkeratosis]	[32] Abscesses	[58] Other: *[Max. 30characters and no space]*
[6] Pallor [Pale]	[33] Cracks	[59] Not Sure / Not Done
[7] Hyperaemia/congestion	[34] Loss of papillae [dorsal tongue only]	Bones & Hooves
[8] Icterus [yellowish]	[35] Foreign bodies	[60] Fracture / Fisures
[9] Cyanosis [bluish]	[36] Opacity [Cornea]	[61] Thickening
[10] Haemorrhages	[37] Perforation	[62] Stress lines [applies to hooves]
[11] Non-exudative nodules/granules	[38] Prolapse [a]	[63] White line disorder: Serum tinged [soles only]
[12] Exudative nodules/granules	[39] Other: *[Max. 30characters no space]*	[64] White line disorder: Blood tinged [soles only]
[13] Non-exudative raised clumps of hair	[40] Not Sure / Not Done	[65] Abnormal convex shape causing asymmetry
[14] Exudative raised clumps of hair	Muscles & Abdomen	[66] Abnormal concave shape causing asymmetry
[15] 'Bleeding spots'	*[41]*	[67] Grown out
[16] 'Breathing holes' [warbles]	[42] Swelling/oedema/enlargement	[68] Other: ***[Max. 30characters and no space]***
[17] Myasis	[43] Atrophy [smaller than normal]	[69] Not Sure / Not Done
[18] Other macroscopic ectoparasites	[44] Gangrene	Special Organs: Eye Balls
[19] Blisters [vesicles/ pustules/papules]	[45] Nodules	[70] Sunken
[20] Warts	[46] Abscesses	[71] Protruding
[21] Non-exudative ulcers/erosions	[47] Scabs	*[72]*
[22] Ulcers/erosions [serous exudates]	[48] Fibrotic scars	[73] Internal haemorrhages
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[24] Ulcers/erosions [purulent exudates]	[50] Fistula [serofibrinous exudates]	[75] Thelazia
[25] Ulcers/erosions [haemorrhagic exudates]	[51] Fistula [purulent exudates]	[76] Other: *[Max. 30characters and no space]*
[26] Scabs	[52] Fistula [haemorrhagic exudates]	[77] Not Sure / Not Done

[a] Refers to third eyelid, rectum, vagina, prepuce [penis]…

15. Internal Post-Mortem Examination: Description of lesions

	Normal	Abnormal	ND											
Blood	□	□	□	___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] : _____	___ [ ] : _____					
Oesophagus	□	□	□	___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] : _____	___ [ ] : _____					
Trachea	□	□	□	___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] : _____	___ [ ] : _____					
Brongi [R/L]	□	□	□	___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] : _____	___ [ ] : _____					
Lung [R/L]	□	□	□	___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] : _____	___ [ ] : _____					
Thymus	□	□	□	___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] : _____	___ [ ] : _____					
Heart	□	□	□	___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] : _____	___ [ ] : _____					
Pericardium	□	□	□	___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] : _____	___ [ ] : _____					
Blood vessels [1]	□	□	□	___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] : _____	___ [ ] : _____					
Thoracic muscle	□	□	□	___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] : _____	___ [ ] : _____					
Thoracic lymph nodes	□	□	□	___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] : _____	___ [ ] : _____					
Diaphragm	□	□	□	___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] : _____	___ [ ] : _____					
Reticulum	□	□	□	___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] : _____	___ [ ] : _____					
Omasum	□	□	□	___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] : _____	___ [ ] : _____					
Abomasum	□	□	□	___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] : _____	___ [ ] : _____					
Rumen	□	□	□	___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] : _____	___ [ ] : _____					
Liver	□	□	□	___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] : _____	___ [ ] : _____					
Gall bladder	□	□	□	___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] : _____	___ [ ] : _____					
Bile ducts	□	□	□	___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] : _____	___ [ ] : _____					
Spleen	□	□	□	___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] : _____	___ [ ] : _____					
				___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] : _____	___ [ ] : _____					
				___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] : _____	___ [ ] : _____					
				___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] : _____	___ [ ] : _____					
				___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] : _____	___ [ ] : _____					
				___ [ ]	___ [ ]	___ [ ]	___ [ ]	___ [ ] : _____	___ [ ] : _____					

[1] [i.e. aorta/cava/ pulmonary]

If abnormal, select from list below. For each body part you may select as many skin lesions / Muscular lesions / Bones, Hooves lesions / Special Organ lesions as you see. For each lesion that you enter you must enter a combined score. The first digit on the score indicates the **location of the lesion**: 1 = Right or dorsal / 2 = Left or ventral; 3 = Bilateral [Both RL or DV]; 4 = Not applicable because the body part is not bilateral; 5 = Not sure, not done. The second digit in the score indicates the **extent of the lesion**: 1 = Focal ; 2 = Multi-focal; 3 = Diffuse; 4 = Mixture of 1 & 2 ; 5 = Mixture of 1 & 3; 6 = Mixture of 2 & 3; 7 = Not sure, not done. The third digit in the score indicates **whether the lesion is located in the capsule of the organ or not**: 1 = Only capsule is affected; 2 = Both capsule and parenchyma are affected; 3 = Only parenchyma is affected; 4 = not applicable because the organ/tissue does not have capsule; 5 = Not sure, not done.

e.g. If you have multi-focal abscesses in the right lung, and a single abscess in the left lung, you would enter code = 344.

[1] Oedema	[16] Atrophy	[31] Emphysema [air accumulation in lung]
[2] Swelling / enlargement / hyperplasia	[17] Stenosis	[32] Thrombus
[3] Pallor	[18] Obstruction	[33] Acute pneumonia
[4] Hyperaemia / congestion	[19] Compression	[34] Chronic pneumonia
[5] Icterus	[20] Foreign bodies	[35] Serofibrinous pericarditis
[6] Cyanosis / methaemoglobinaemia	[21] Perforation	[36] Fibrinous / fibrinopurulent meningitis
[7] Haemorrhages	[22] Prolapse	[37] Prominent white pulp [spleen]
[8] Granulomas / nodules	[23] Displacement	[38] Endoparasites [cyst-like]
[9] Cavities	[24] Torsion	[39] Endoparasites [worm-like]
[10] Ulcers	[25] White spots [kidneys]	[40] Endoparasites [leaf-like]
[11] Erosions	[26] Infarcts	[41] Endoparasites [nodule-like]
[12] Adhesions	[27] Necrosis	[42] Endoparasites [other]
[13] Deposit of fibrin	[28] Fibrous	[43] Other: *[Max. 30characters and no space]*
[14] Calcifications	[29] Darker/reddish areas [i.e. lung]	[ND] Not sure / not done
[15] Abscesses	[30] Consolidation [hard, heavy areas]	

15. Internal Post-Mortem Examination [Continued]: Description of lesions

	Normal	Abnormal	ND						
Pancreas	☐	☐	☐	___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____	___[ ]:_____
Small intestine	☐	☐	☐	___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____	___[ ]:_____
Large intestine	☐	☐	☐	___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____	___[ ]:_____
Abdominal muscle	☐	☐	☐	___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____	___[ ]:_____
Mesenteric lymph nodes	☐	☐	☐	___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____	___[ ]:_____
Kidney [R/L]	☐	☐	☐	___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____	___[ ]:_____
Bladder	☐	☐	☐	___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____	___[ ]:_____
Uterus	☐	☐	☐	___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____	___[ ]:_____
Testicles [R/L]	☐	☐	☐	___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____	___[ ]:_____
Brain	☐	☐	☐	___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____	___[ ]:_____
Cerebellum	☐	☐	☐	___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____	___[ ]:_____
Spinal Cord	☐	☐	☐	___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____	___[ ]:_____
Shoulder joint [R/L]	☐	☐	☐	___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____	___[ ]:_____
Elbow Joint [R/L]	☐	☐	☐	___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____	___[ ]:_____
Carpal joint [R/L]	☐	☐	☐	___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____	___[ ]:_____
Fetlock joint forelimb [R/L]	☐	☐	☐	___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____	___[ ]:_____
Hip joint [R/L]	☐	☐	☐	___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____	___[ ]:_____
Stifle Joint [R/L]	☐	☐	☐	___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____	___[ ]:_____
Tarsal joint [R/L]	☐	☐	☐	___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____	___[ ]:_____
Fetlock joint HL [R/L]	☐	☐	☐	___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____	___[ ]:_____
				___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____	___[ ]:_____
				___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____	___[ ]:_____
				___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____	___[ ]:_____
				___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____	___[ ]:_____
				___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____	___[ ]:_____

If abnormal, select from list below. For each body part you may select as many skin lesions / Muscular lesions / Bones, Hooves lesions / Special Organ lesions as you see. For each lesion that you enter you must enter a combined score. The first digit on the score indicates the **location of the lesion**: 1 = Right or dorsal / 2 = Left or ventral; 3 = Bilateral [Both RL or DV]; 4 = Not applicable because the body part is not bilateral; 5 = Not sure, not done. The second digit in the score indicates the **extent of the lesion**: 1 = Focal ; 2 = Multi-focal; 3 = Diffuse; 4 = Mixture of 1 & 2 ; 5 = Mixture of 1 & 3; 6 = Mixture of 2 & 3; 7 = Not sure, not done. The third digit in the score indicates **whether the lesion is located in the capsule of the organ or not**: 1 = Only capsule is affected; 2 = Both capsule and parenchyma are affected; 3 = Only parenchyma is affected; 4 = not applicable because the organ/tissue does not have capsule; 5 = Not sure, not done.

e.g. If you have multi-focal abscesses in the right lung, and a single abscess in the left lung, you would enter code = 344.

[1] Oedema	[16] Atrophy	[31] Emphysema [air accumulation in lung]
[2] Swelling / enlargement / hyperplasia	[17] Stenosis	[32] Thrombus
[3] Pallor	[18] Obstruction	[33] Acute pneumonia
[4] Hyperaemia / congestion	[19] Compression	[34] Chronic pneumonia
[5] Icterus	[20] Foreign bodies	[35] Serofibrinous pericarditis
[6] Cyanosis / methaemoglobinaemia	[21] Perforation	[36] Fibrinous / fibrinopurulent meningitis
[7] Haemorrhages	[22] Prolapse	[37] Prominent white pulp [spleen]
[8] Granulomas / nodules	[23] Displacement	[38] Endoparasites [cyst-like]
[9] Cavities	[24] Torsion	[39] Endoparasites [worm-like]
[10] Ulcers	[25] White spots [kidneys]	[40] Endoparasites [leaf-like]
[11] Erosions	[26] Infarcts	[41] Endoparasites [nodule-like]
[12] Adhesions	[27] Necrosis	[42] Endoparasites [other]
[13] Deposit of fibrin	[28] Fibrous	[43] Other: *[Max. 30characters and no space]*
[14] Calcifications	[29] Darker/reddish areas [i.e. lung]	[ND] Not sure / not done
[15] Abscesses	[30] Consolidation [hard, heavy areas]	

16. Internal Post-Mortem Examination: Description of Ruminal Content

Describe whether the rumen is well filled or not well filled:

☐ The rumen is well filled ☐ The rumen is not well filled ☐ ND

Describe stomach contents [Please note that presence of parasites has already been considered elsewhere]

	Normal	Abnormal	ND					
Reticulum	☐	☐	☐	___	___	___	___ :_____	___ :_____
Omasum	☐	☐	☐	___	___	___	___ :_____	___ :_____
Abomasum	☐	☐	☐	___	___	___	___ :_____	___ :_____
Rumen	☐	☐	☐	___	___	___	___ :_____	___ :_____

[1] Dry stomach content	[3] Haemorrhagic stomach content	[ND] Not sure, not done
[2] Foamy stomach content	[4] Other: *[Max. 30characters and no space]*	

17. Description of Intestinal Content: Faeces

Consistency: Hard☐ Normal☐ Diarrhoea☐ ND☐

Type of Diarrhoea: No diarrhoea present ☐ Diarrhoea present; enter combined score as explained below ☐ NA☐

Use a combined score to define the type of diarrhoea, using the table on the right:

For example, if the diarrhoea is moderate, haemorrhagic and foul-smelling, the code = 243

	First Digit = Severity	Second Digit = Type	Third digit = Odour
1	Mild	Watery	Normal
2	Moderate	Catarrhal	Sour
3	Severe	Mucohaemorrhagic	Foul-smelling
4	Don't Know	Haemorrhagic	Don't Know
5		Other	
6		Don't Know	

Comb Score

18. Internal Post-Mortem Examination: Description of Fluids and Exudates

	Normal	Abnormal	ND						
Tracheal	☐	☐	☐	___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____	___[ ]:_____
Brongi [R/L]	☐	☐	☐	___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____	___[ ]:_____
Pericardial	☐	☐	☐	___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____	___[ ]:_____
Pleural [R/L]	☐	☐	☐	___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____	___[ ]:_____
Thoracic	☐	☐	☐	___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____	___[ ]:_____
Peritoneal	☐	☐	☐	___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____	___[ ]:_____
Bile	☐	☐	☐	___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____	___[ ]:_____
Cerebrospinal	☐	☐	☐	___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____	___[ ]:_____
Urine	☐	☐	☐	___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____	___[ ]:_____
				___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____	___[ ]:_____
				___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____	___[ ]:_____
				___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____	___[ ]:_____
				___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____	___[ ]:_____
				___[ ]	___[ ]	___[ ]	___[ ]	___[ ]:_____	___[ ]:_____

If abnormal, select from list below. For each body part you may select as many lesions as you see. For each lesion that you enter you must enter a score that indicates the **location of the lesion**: 1 = Right or dorsal / 2 = Left or ventral; 3 = Bilateral [Both RL or DV]; 4 = Not applicable because the body part is not bilateral; 5 = Not sure, not done.

Urine & Bile	[11] Fibrinopurulent discharge	Others
[1] Water coloured urine	[12] Mucus discharge	[22] Increased fluid content
[2] Reddish-tinged [urine/bile]	[13] Mucopurulent discharge	[23] Serous fluid
[3] Brownish-tinged urine	[14] Purulent discharge	[24] Serofibrinous fluid
[4] Dark brown-tinged bile	[15] Blood-tinged discharge	[25] Fibrinopurulent fluid
[5] Increased consistency [>>density; bile/urine]	[16] Foamy discharge	[26] Mucus discharge
[6] Sand [Bile]	[17] Blood-tinged foamy discharge	[27] Mucopurulent discharge
[7] Other: *[Max. 30characters and no space]*	[18] Free blood	[28] Purulent fluid
[8] Not sure, not done	[19] Rumen content	[29] Blood-tinged
Tracheal & Brongi	[20] Other: *[Max. 30characters and no space]*	[30] Free blood
[9] Serous discharge	[21] Not sure, not done	[31] Other: *[Max. 30characters and no space]*
[10] Serofibrinous discharge		[32] Not sure, not done

19. Minimum Required Sample Collection

Select types from list below and enter Barcode ID. The minimum number of samples required is shown in brackets. You have the option of collecting additional samples:

Routine MKS smear [n = 2]:

ID ☐ ND☐

Numb ☐ NA☐

Faecal Sample [n = 2]:

ID ☐ ND☐

Numb ☐ NA☐

Tick result of anthrax smear prior to opening the carcass:

☐ Positive

☐ Negative

☐ ND

Routine MNS smear [n = 2]:

ID ☐ ND☐

Numb ☐ NA☐

20. Congenital Disorders

1. Over the course of the clinical examination, have you observed any congenital disorder?

☐ Yes ☐ No

If yes, fill in the Congenital Disorders form for Calves.

21. Additional Samples

Are you collecting additional blood, organ or tissue samples?

☐ Yes

☐ No. Sample collection is not needed because cause of death is obvious upon animal inspection, and there is no apparent disease.

☐ I should have collected additional samples but it was not possible

Are you collecting fluid or exudate samples?

☐ Yes

☐ No. Sample collection is not needed because cause of death is obvious upon animal inspection, and there is no apparent disease.

☐ I should have collected additional samples but it was not possible

22. Additional Samples From Post-Mortem Examinations

Date: **Time:** **Visit ID:** **AHA ID:**

Sample Collection I: Samples from Organs and Tissues

	Body Part		Sample Code		Barcode ID Sample		N Samples
BP		C		ID		N	1
BP		C		ID		N	1
BP		C		ID		N	1
BP		C		ID		N	1
BP		C		ID		N	1
BP		C		ID		N	1
BP		C		ID		N	1
BP		C		ID		N	1
BP		C		ID		N	1
BP		C		ID		N	1
BP		C		ID		N	1
BP		C		ID		N	1
BP		C		ID		N	1
BP		C		ID		N	1

Codes for Body Parts

[1] Blood
[2] Multiple body parts
[3] Face
[4] Head
[5] Ear
[6] Conjunctiva
[7] Cornea
[8] Sclera
[9] Eyelid
[10] Third eyelid
[11] Muzzle
[12] Nostrils
[13] Lips
[14] Gums
[15] Palate
[16] Tongue
[17] Mouth floor [interior]
[18] Cheeks [interior]
[19] Inter-mandibular sp [ext]
[20] Parotid lymph node
[21] Neck [DV]
[22] Lateral neck
[23] Supra-scapular LN
[24] Forelimb
[25] Coronary band FL
[26] Hoof forelimb
[27] Inter-digital FL
[28] Sole FL
[29] Thorax [DV]
[30] Bone marrow ribs
[31] Lateral thorax
[32] Abdomen [DV]
[33] Umbilicus
[34] Udder
[35] Testis
[36] Penis
[37] Lateral abdomen
[38] Liver
[39] Kidney
[40] Spleen
[41] Pre-crural LN
[42] Hindlimb
[43] Coronary Band HL
[44] Hoof HL
[45] Inter-digital HL
[46] Sole HL
[47] Perianal area
[48] Anus
[49] Vulva
[50] Tail

For each sample you collect, choose the exact location of the sample. For example, if you are collecting pus from an abscess located in the neck, you may select option 1 if you are sampling from below the integument (e.g. muscular abscess), or option 3 if the abscess is in the integument (e.g. skin). Then enter the Barcode ID of each sample. You have the option of collecting samples from multiple body parts simultaneously.

Sample Codes for Tissue Types 1: Samples from Soft Organs and Tissues (i.e. Below the integument)

[1] Bacteriology [Swab]	[4] Virology or PCR tissue/material [-80°C]	[7] Toxicology tissue/ material [-80°C]
[2] Virology or PCR [Swab]	[5] **NO LONGER IN USE**	[8] Histopathology [10% Formalin]
[3] Bacteriology tissue/material [-80°C]	[6] Impression smear, aspirate, etc. [Slide]	[9] Endoparasites [70% Ethanol]

Sample Codes for Tissue Types 2: Samples from Jugular Vein Blood

[10] Bacteriology [Swab]	[12] Heparinised vacutainer: Bacteriology [-80°C]	[14] **NO LONGER IN USE**
[11] Virology or PCR [Swab]	[13] **NO LONGER IN USE**	

Sample Codes for Tissue Types 3: Samples from Integument (i.e. Skin, hair, hooves, mucous membranes)

[15] Bacteriology [Swab]	[19] **NO LONGER IN USE**	[23] Deep scrape [Slide / Dermapack]
[16] Virology or PCR [Swab]	[20] Impression smear, aspirate, etc. [Slide]	[24] Toxicology [-70°C]
[17] Bacteriology tissue/material [-80°C]	[21] Snip [Slide]	[25] Histopathology [10% Formalin]
[18] Virology or PCR tissue [-70°C]	[22] Superficial scrape [Slide / Dermapack]	[26] Ectoparasites [70% Ethanol]

22. Additional Samples From Post-Mortem Examinations

Date: **Time:** **Visit ID:** **AHA ID:**

Sample Collection I: Samples from Organs and Tissues

	Body Part		Sample Code		Barcode ID Sample		N Samples
BP		C		ID		N	1
BP		C		ID		N	1
BP		C		ID		N	1
BP		C		ID		N	1
BP		C		ID		N	1
BP		C		ID		N	1
BP		C		ID		N	1
BP		C		ID		N	1
BP		C		ID		N	1
BP		C		ID		N	1
BP		C		ID		N	1
BP		C		ID		N	1
BP		C		ID		N	1
BP		C		ID		N	1

Codes for Body Parts

[51] Oesophagus
[52] Lung
[53] Thymus
[54] Heart
[55] Blood vessels [1]
[56] Thoracic lymph nodes
[57] Diaphragm
[58] Reticulum
[59] Omasum
[60] Abomasum
[61] Rumen
[62] Gall bladder
[63] Pancreas
[64] Small intestine
[65] Large Intestine
[66] Mesenteric lymph nodes
[67] Bladder
[68] Uterus
[69] Brain
[70] Cerebellum
[71] Spinal cord

[1] [i.e. aorta/cava/pulmonary]

For each sample you collect, choose the exact location of the sample. For example, if you are collecting pus from an abscess located in the neck, you may select option 1 if you are sampling from below the integument (e.g. muscular abscess), or option 3 if the abscess is in the integument (e.g. skin). Then enter the Barcode ID of each sample. You have the option of collecting samples from multiple body parts simultaneously.

Sample Codes for Tissue Types 1: Samples from Soft Organs and Tissues (i.e. Below the integument)

[1] Bacteriology [Swab]	[4] Virology or PCR tissue/material [-80°C]	[7] Toxicology tissue/ material [-80°C]
[2] Virology or PCR [Swab]	[5] **NO LONGER IN USE**	[8] Histopathology [10% Formalin]
[3] Bacteriology tissue/material [-80°C]	[6] Impression smear, aspirate, etc. [Slide]	[9] Endoparasite [70% Ethanol]

Sample Codes for Tissue Types 2: Samples from Jugular Vein Blood

[10] Bacteriology [Swab]	[12] Heparinised vacutainer: Bacteriology [-80°C]	[14] **NO LONGER IN USE**
[11] Virology or PCR [Swab]	[13] **NO LONGER IN USE**	

Sample Codes for Tissue Types 3: Samples from Integument (i.e. Skin, hair, hooves, mucous membranes)

[15] Bacteriology [Swab]	[19] **NO LONGER IN USE**	[23] Deep scrape [Slide / Dermapack]
[16] Virology or PCR [Swab]	[20] Impression smear, aspirate, etc. [Slide]	[24] Toxicology [-70°C]
[17] Bacteriology tissue/material [-80°C]	[21] Snip [Slide]	[25] Histopathology [10% Formalin]
[18] Virology or PCR tissue [-70°C]	[22] Superficial scrape [Slide / Dermapack]	[26] Ectoparasites [70% Ethanol]

22. Additional Samples From Post-Mortem Examinations

Date: **Time:** **Visit ID:** **AHA ID:**

Sample Collection II: Exudates, Fluids, Discharges, Material from Body Cavities and Joints

	Body Part		Sample Code		Barcode ID Sample		N Samples
BP		C		ID		N	1
BP		C		ID		N	1
BP		C		ID		N	1
BP		C		ID		N	1
BP		C		ID		N	1
BP		C		ID		N	1
BP		C		ID		N	1
BP		C		ID		N	1
BP		C		ID		N	1
BP		C		ID		N	1
BP		C		ID		N	1
BP		C		ID		N	1
BP		C		ID		N	1
BP		C		ID		N	1

Codes for Body Parts

[72] Ocular
[73] Nasal
[74] Ear
[75] Pericardial
[76] Pleural
[77] Thoracic
[78] Shoulder joint
[79] Elbow Joint
[80] Carpal Joint
[81] Fetlock joint forelimb
[82] Peritoneal
[83] Umbilicus
[84] Udder Content
[85] Cerebrospinal
[86] Hip Joint
[87] Stifle joint
[88] Tarsal Joint
[89] Fetlock joint hindlimb
[90] Vaginal
[91] Prepucial
[92] Urine
[93] Tracheal
[94] Brongi
[95] Bile

For each sample you collect, choose the exact location of the sample. For example, you may be collecting pus from the shoulder joint through an aspirate, or you may be collecting purulent ocular discharge or exudates from the pericardial sac. Then enter the Barcode ID for each sample.

Sample Code

[27] Bacteriology [Swab]	[30] Virology or PCR exudate/fluid/other [-80°C]	[33] Toxicology [-80°C]
[28] Virology or PCR [Swab]	[31] **NO LONGER IN USE**	[34] **NO LONGER IN USE**
[29] Bacteriology exudate/fluid/other [-80°C]	[32] Impression smear, aspirate, etc. [Slide]	

23. Pictures

It is compulsory that you take AT LEAST one picture from each lesion for later validation of the current classification of lesions. You must save your pictures electronically, but a paper copy of the pictures is also required. It is compulsory that you attach a copy of your pictures here:

Section:
Body Part:
Lesion code:
Picture ID:

Section:
Body Part:
Lesion code:
Picture ID:

Section:
Body Part:
Lesion code:
Picture ID:

Section:
Body Part:
Lesion code:
Picture ID:

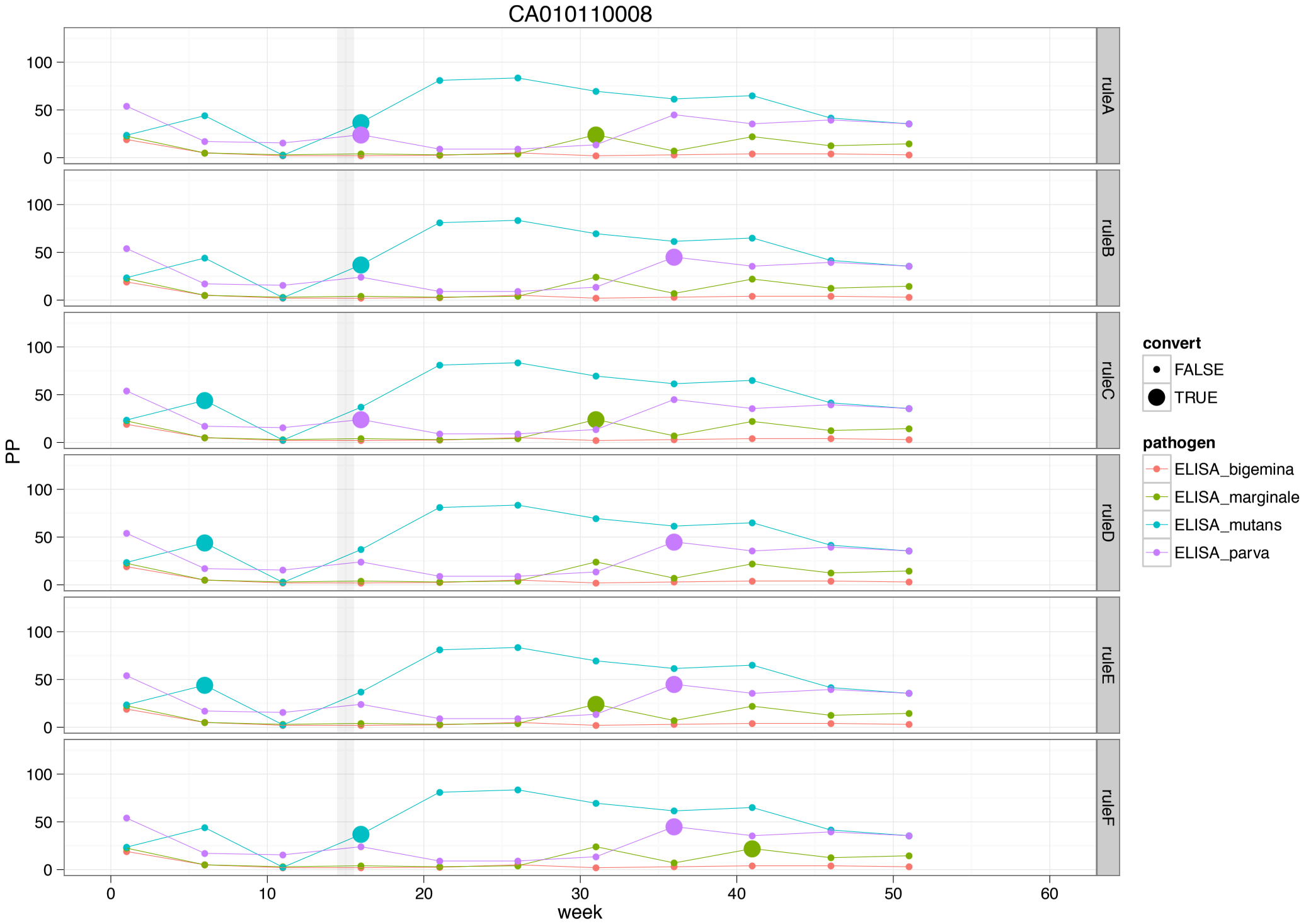
Section:
Body Part:
Lesion code:
Picture ID:

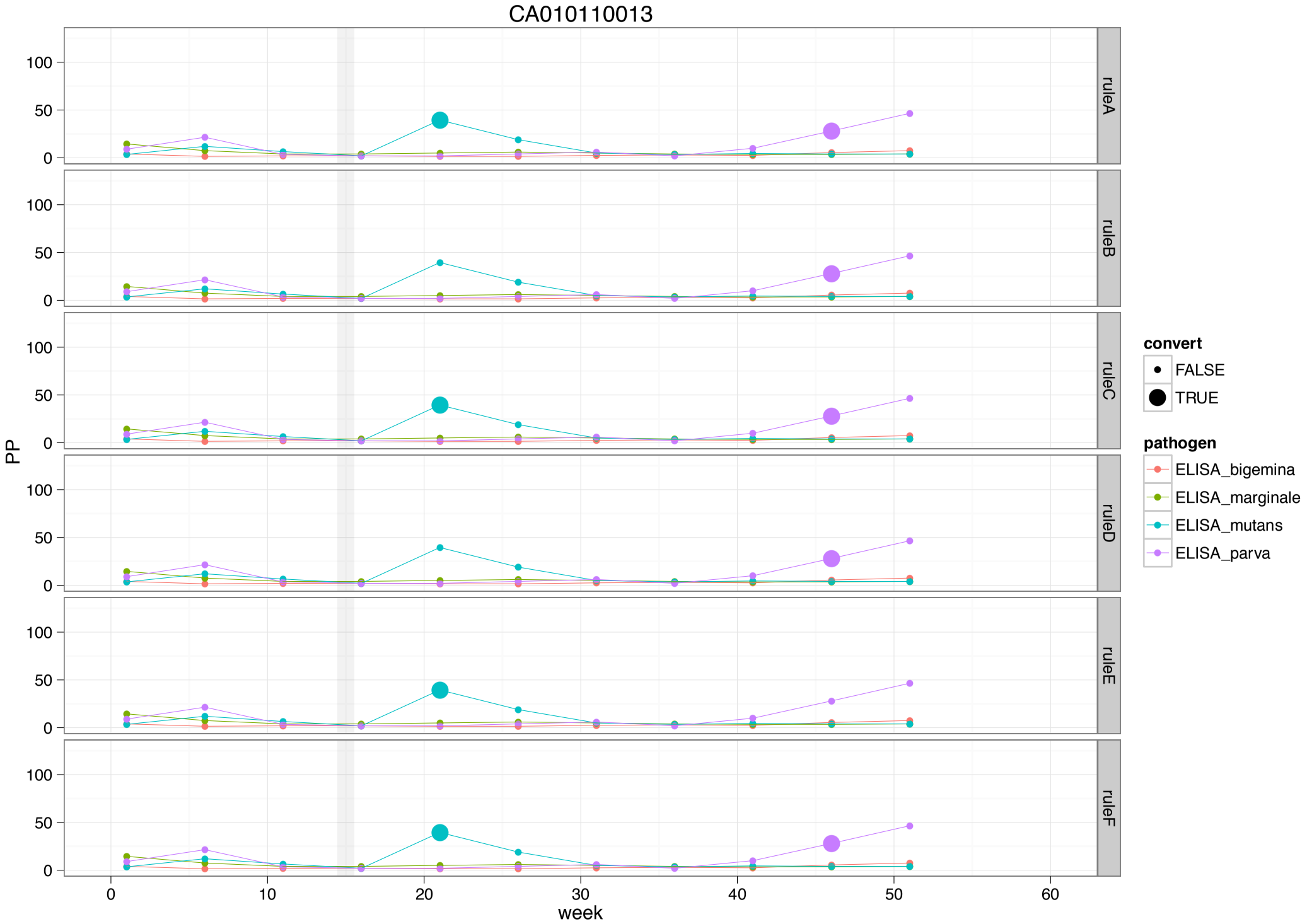
Section:
Body Part:
Lesion code:
Picture ID:

Appendix G

Examples of ELISA results by calf

These plots show examples of the ELISA results by calf for the four tick borne diseases tested in this manner at every visit. The points of seroconversion by the different rules are marked by large circles. This is a selection of plots that allow comparison of the performance of the different rules.


CA010110008
ruleA
ruleB
ruleC
ruleD
ruleE
ruleF
PP
week
0
10
20
30
40
50
60
100
50
0
convert
FALSE
TRUE
pathogen
ELISA_bigemina
ELISA_marginale
ELISA_mutans
ELISA_parva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ruleD
ruleE
ruleF
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pathogen
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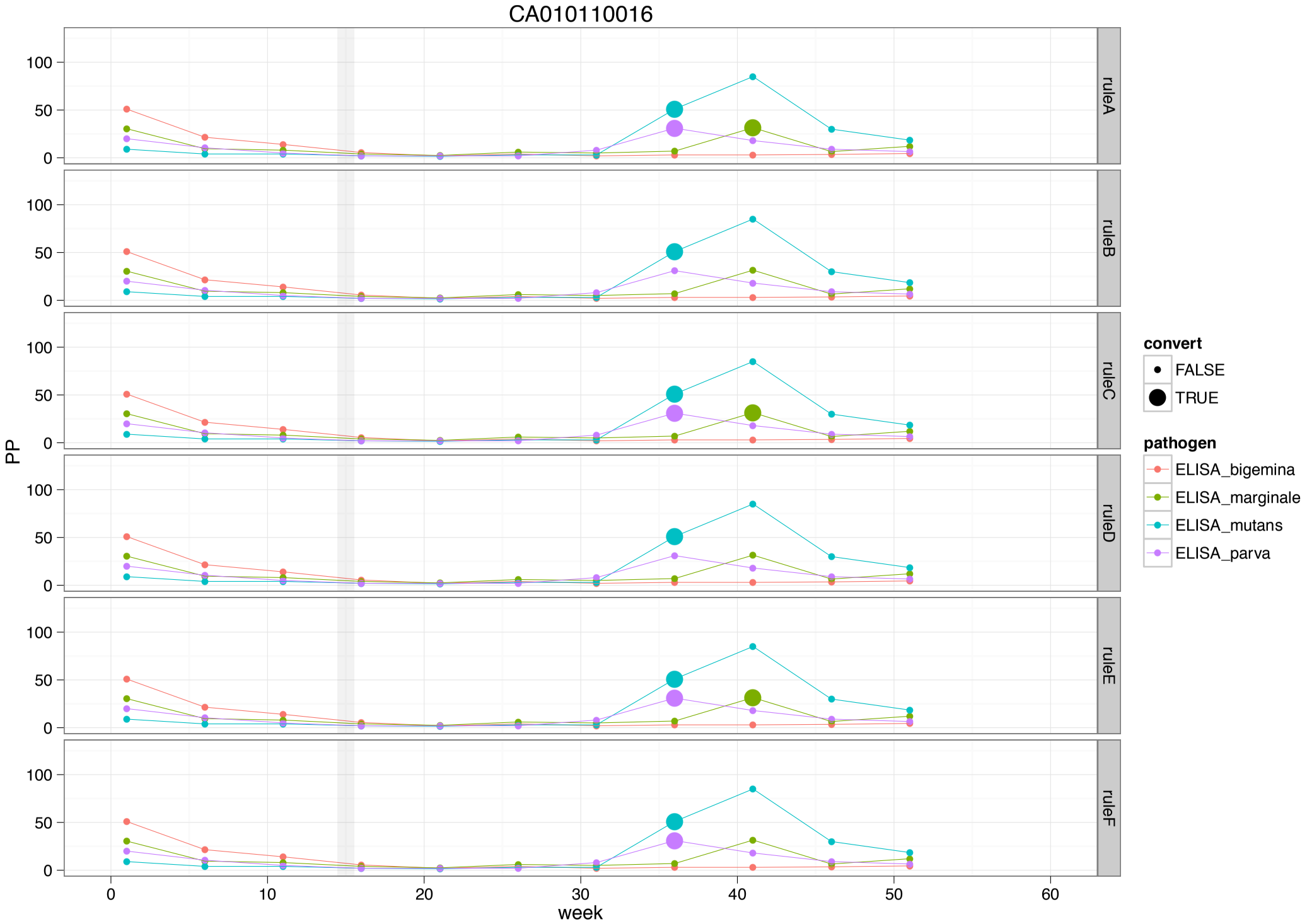
ELISA_marginale
ELISA_mutans
ELISA_parva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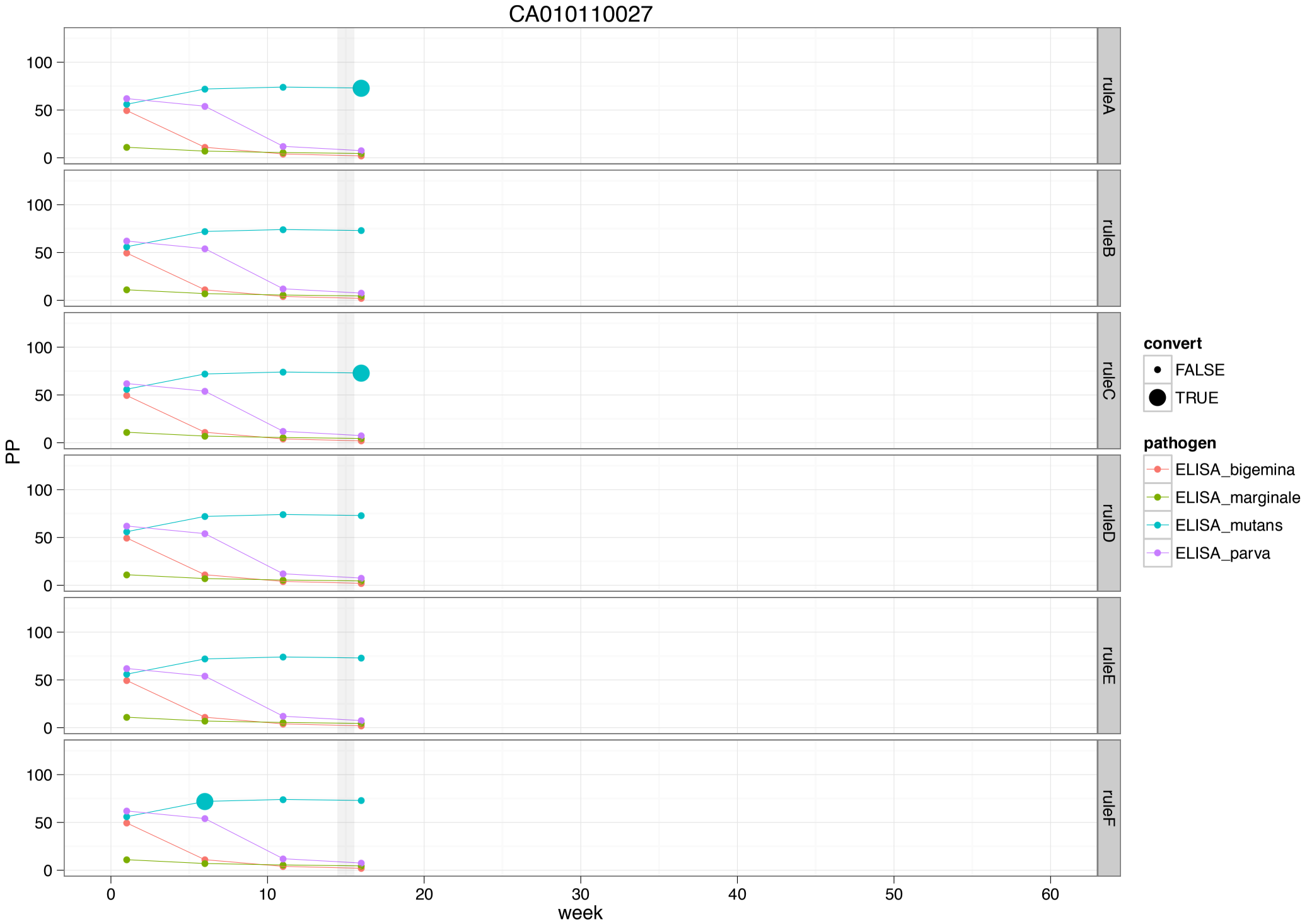
pathogen
ELISA_bigemina
ELISA_marginale
ELISA_mutans
ELISA_parva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week
convert
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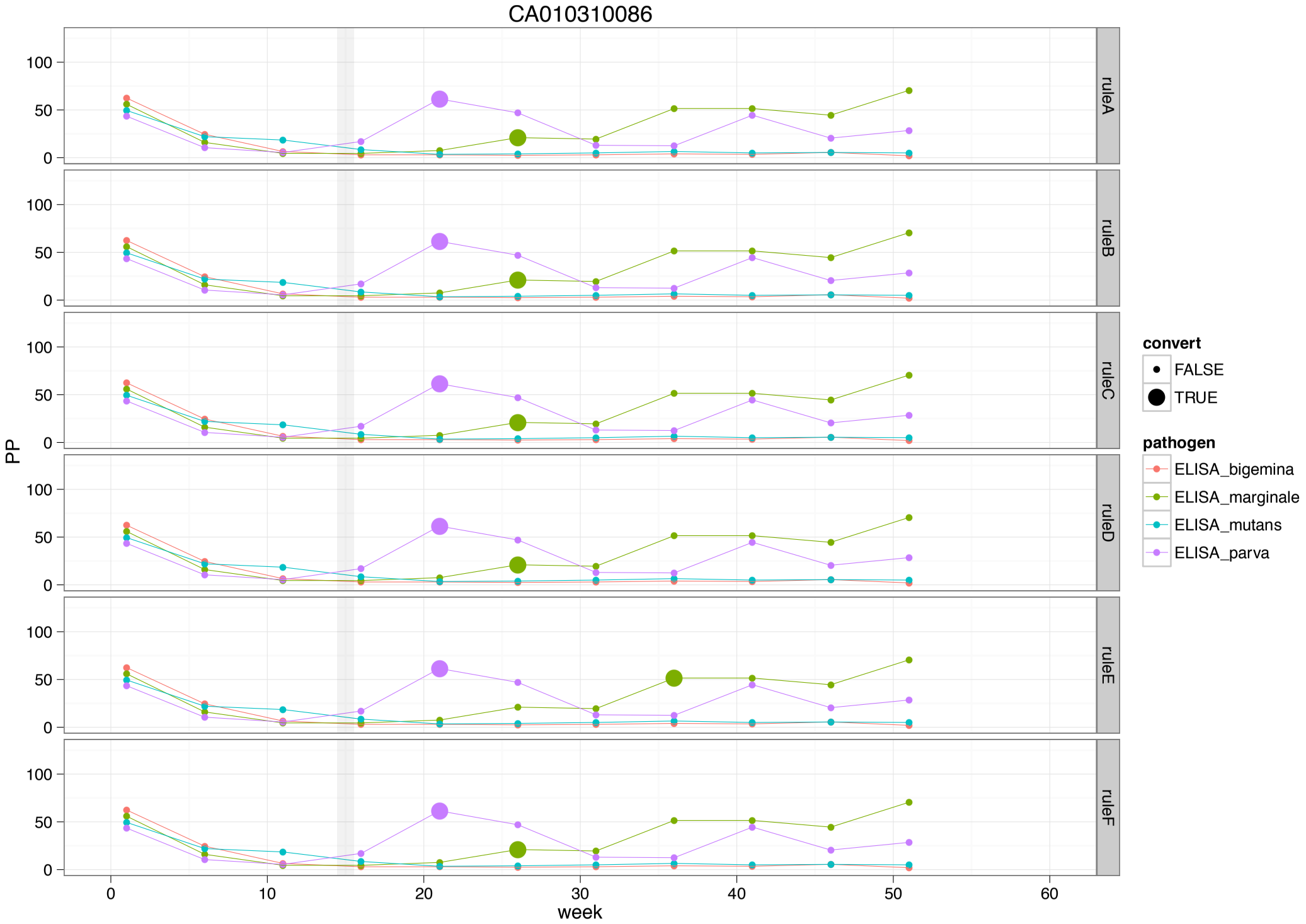
pathogen
ELISA_bigemina
ELISA_marginale
ELISA_mutans
ELISA_parva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convert
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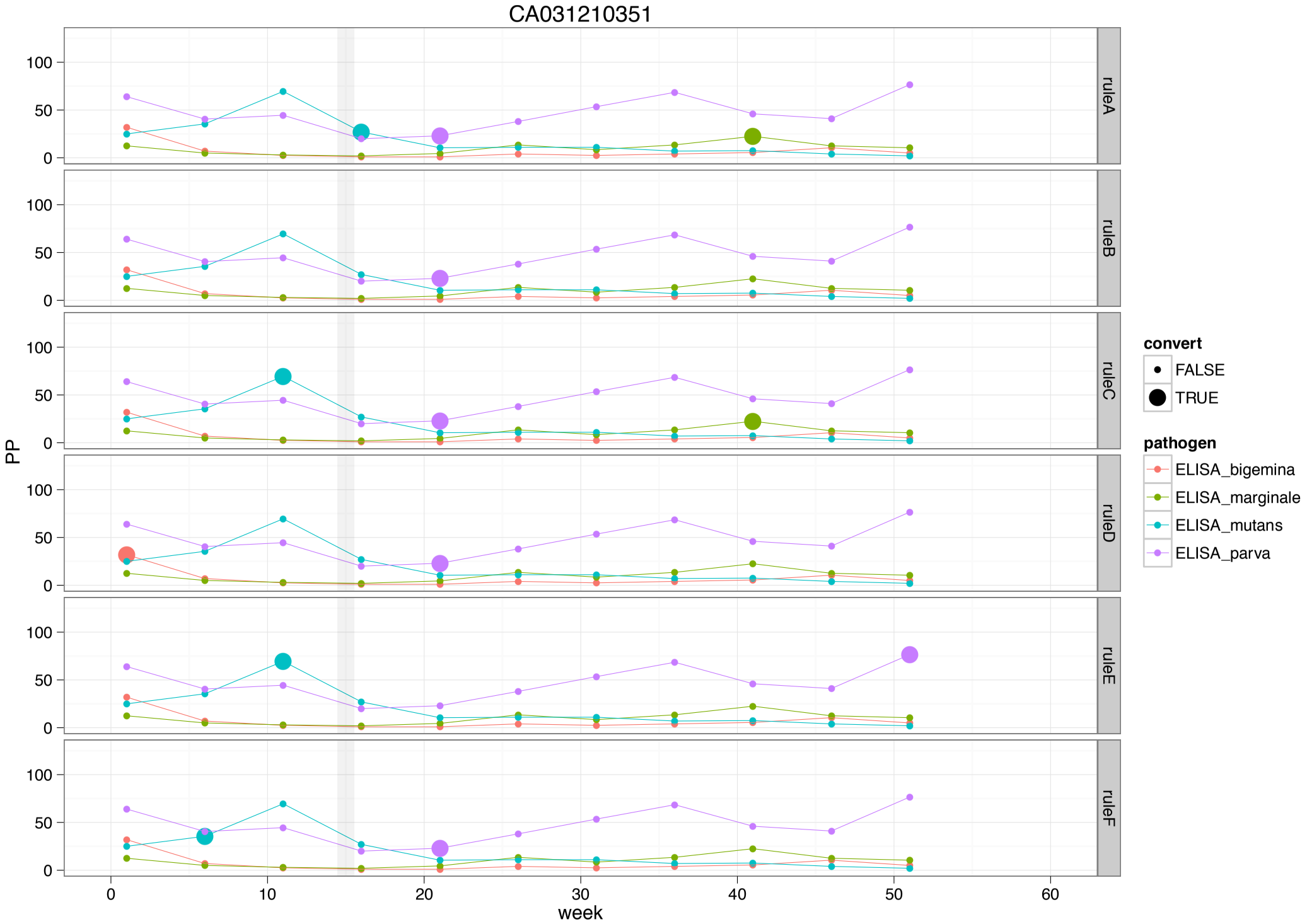
TRUE
pathogen
ELISA_bigemina
ELISA_marginale
ELISA_mutans
ELISA_parva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ruleB
ruleC
ruleD
ruleE
ruleF
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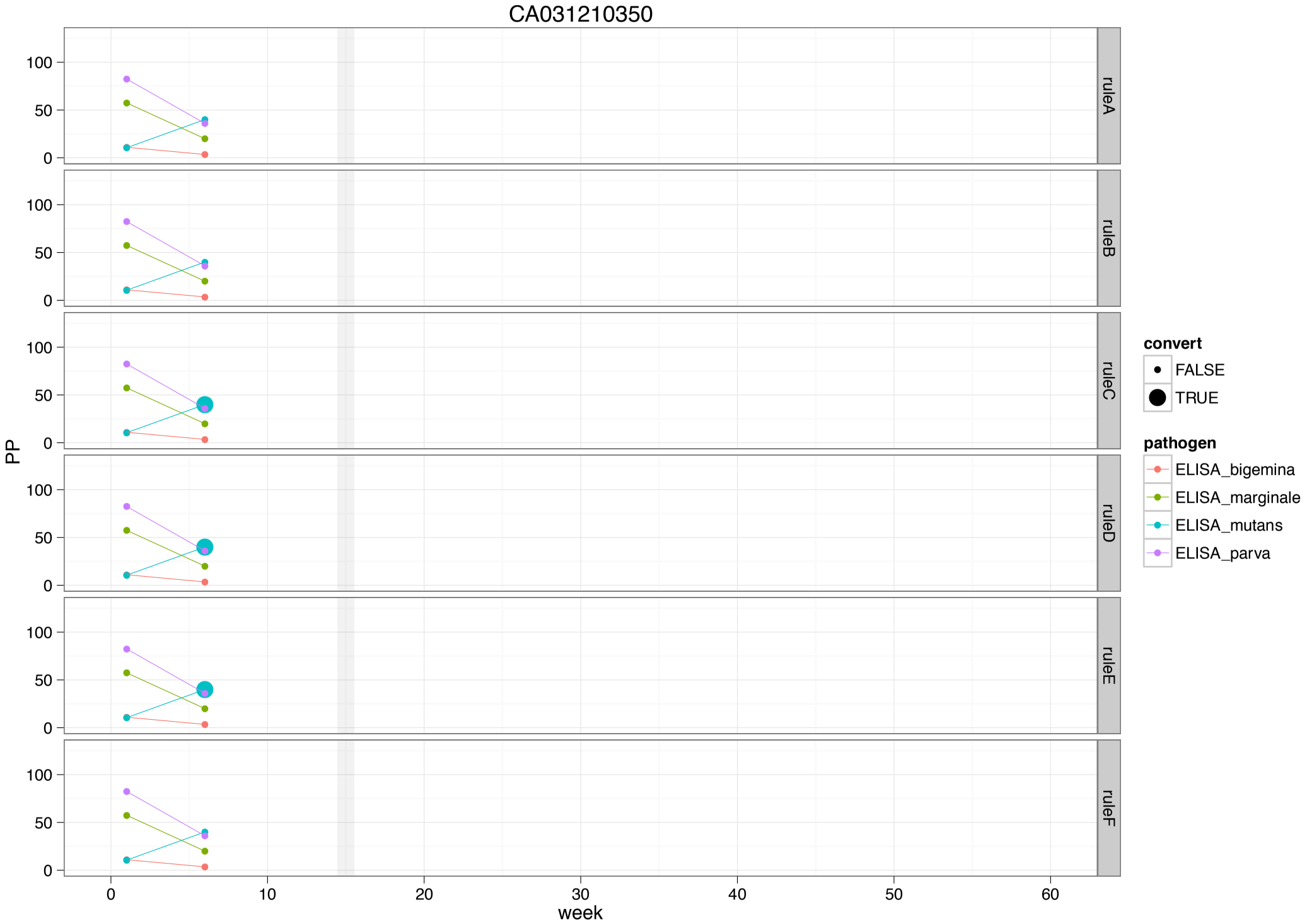
convert
FALSE
TRUE
pathogen
ELISA_bigemina
ELISA_marginale
ELISA_mutans
ELISA_parva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ruleB
ruleC
ruleD
ruleE
ruleF
PP
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50
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0
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60
week
convert
FALSE
TRUE
pathogen
ELISA_bigemina
ELISA_marginale
ELISA_mutans
ELISA_parva

Appendix H

Theileria species and their vectors in the study cohort

H.1 Variables associated with hazard of seroconversion

Table H.1: Exposures screened for association with the hazard of seroconversion to *T. parva* and *T. mutans*

Exposure	Description	Data type
FARM		
Agroecological zone	The 5 zones making up the study site upon which stratification was based.	Categorical (1-5)
Sublocation	Smallest administrative unit in Kenya from which calves were sampled	Categorical (1-20)
Elevation	The elevation of the homestead	Continuous (per 50m) & categories (<1198m, 1199-1238m, 1239-1269m, >1269

Continued on next page

Table H.1 – continued from previous page

Exposure	Description	Data type
Farm size	The total number of acres owned or rented	Continuous (acres)
Mean NDVI	The mean normalised density vegetation index (NDVI)	Continuous
Adult female cattle	The total number of female cattle in the calf's herd	
Tropical livestock units	The total stock kept by the homestead corrected for animal type	Continuous (TLU)
FARMER		
Farmer sex	The gender of the owner of the calf	Categorical (male (1) / female (2))
Farmer age	The age of the owner of the calf at recruitment	Continuous (per 10 years)
Farmer occupation	Whether the calf owners main occupation was farming	Categorical (yes/no)
Farmer training	Whether the owner of the calf had received technical training	Categorical (yes/no)
Farmer education	Whether the farmer had been educated to at least primary level	Categorical (yes/no)
HUSBANDRY		
Water provision for herd	The method of providing water to cattle	Categorical (at homestead / driven to water)
Supplementary feeding	Whether the farmer reported supplementary feeding in the herd (usually crop residue or cut grass)	Categorical (yes/no)
Acaricide use	Whether the farmer reported using acaricides on the herd	Categorical (yes/no)
Vaccine use	Whether the farmer reported using vaccines on the herd	Categorical (yes/no)
Veterinary support	Whether the farmer reported accessing veterinary support for the herd	Categorical (yes/no)
Tick control	The number of times the farmer reported using tick control during the time the calf was in the study	Count

Continued on next page

Table H.1 – continued from previous page

Exposure	Description	Data type
Worm control	The number of times the farmer reported using anthelminthcs during the time the calf was in the study	Count
Antibiotics application	The number of times the farmer reported using antibiotics during the time the calf was in the study	Count
Vaccine application	The number of times the farmer reported using vaccines during the time the calf was in the study	Count
Protozoal control application	The number of times the farmer reported using anti-protozoal drugs during the time the calf was in the study	Count
CALF AT BIRTH		
Calf Sex	The gender of the calf	Categorical (male (1) / female (2))
Genetic introgression	Level of European taurine genetic introgression calculated from SNP analysis	Categorical (low $<$ 0.0125, moderate to high $>$0.0125)
Birth weight	The weight of the calf at the recruitment visit	Continuous (Kg) & categories ($<$20Kg, 20-25Kg, $>$25Kg)
Dam girth at birth	The heart girth measurement of the dam taken at the recruitment visit	Continuous (cm)
Milking post calving	Whether the farmer reported milking the dam immediately after birth before the calf sucked	Categorical (yes/no)
Dam ELISA result	The results of ELISA testing on serum taken from the dam at the recruitment visit.	Continuous (percentage positivity, PP)
Dam ELISA positive	Whether the ELISA PP for the serum sample from the dam at recruitment was more than the standard cut-off	Categorical (yes/no) For TBDs, T. parva, T. mutans, A. marginale, B. bigemina
TIME DEPENDENT		

Continued on next page

Table H.1 – continued from previous page

Exposure	Description	Data type
NDVI	The normalised density vegetation index value for the data collected closest in time to the routine visit date	Continuous
NDVI 5 week lag	The normalised density vegetation index value for the data collected closest in time to the previous routine visit date	Continuous
Dam girth	The heart girth measurement of the dam taken at each routine visit up to weaning of the calf	Continuous (cm)
Dam girth difference	The change in heart girth measurement from the previous to the visit of interest	Continuous (cm)
Dam condition score	The condition score of the dam at the visit	Ordinal categorical
Grazing	Whether the calf was going outside the homestead for grazing with the adult cattle by the visit of interest	Categorical (yes/no)
Suckling	Whether the dam was still suckling the calf	Categorical (yes/no (weaned))
R. appendiculatus	Whether adult *R. appendiculatus* was attached to the calf at the visit of interest	Categorical (yes/no)
A. variegatum	Whether adult *A. variegatum* was attached to the calf at the visit	Categorical (yes/no)
Infection level on microscopy	A semi-quantitative measure of the number of infected erythrocytes (separate assessment for thick and thin smears and for schizonts and piroplasms)	Ordinal categorical (0,1,2,3)

Appendix I

Expression of infectious disease in the cohort

I.1 Clinical signs and the number of times they were observed in the cohort calves

Table I.1: Table summarising the clinical signs recorded and the number of visits in which the sign was observed

Clinical sign	NoVisitRecorded
Rough staring coat	1675
Weight loss / Loss of condition	497
Lethargy	187
Lymph node hyperplasia	138
Pale mucous membranes	138
Weakness	107
Decreased appetite	98
Soiling	88
Shallow rapid breathing	64
Cough	39
Nodular lesions	36
Ocular discharge	36

Continued on next page

Table I.1 – continued from previous page

Clinical sign	NoVisitRecorded
Cachexy / extreme thinness	31
Anorexia	29
Non bloody diarrhoea	27
Wide spread scars / scabs	27
Recumbency	23
Costo abdominal respiration	22
Generalised alopecia	21
Decreased water intake	20
Limping	20
Corneal opacity	19
Potbelly	19
Non foamy nasal discharge	17
Hard faeces (not bloody)	15
Deep laboured breathing	14
Swaying hind quarter	14
Arched back	8
Ulcers / erosions	8
Tenesmus / straining	7
Muscular tremors / twitching	6
Convulsions	5
Increased water intake	5
Unable to swallow food	5
Bloody diarrhoea	4
Hypersensitivity	4
Constipation	3
Excessive salivation / drooling	3
Extended head and neck	3
Food apprehension problems	3
Hard faeces (bloody)	3
Swelling of ventral thorax	3
Circling	2
Joints	2
Lateral positioning of the head	2
Paralysis	2
Icterus	2
Blindness	1

Continued on next page

Table I.1 – continued from previous page

Clinical sign	NoVisitRecorded
Blisters / Vesicles / pustules	1
Excessive bellowing	1
Excessive licking	1
Excessive urination	1
Generalised sloughing of skin	1
Lameness	1
Nystagmus	1
Reddish tinged urine	1
Reduced sensitivity	1
Star gazing	1
Ventral abdomen	1
Wide base stance	1

I.2 Results from MCA and PCA on post-mortem data

Table I.2: Summary of results for dimension 1. PCA on clinical signs associated with post-mortem visits.

	Estimate	p.value
Heartwater	1.53	0.00
ECF	-0.52	0.05
Haemonchosis	-1.09	0.01
	correlation	p.value
LymNum_sysmex	0.80	0.00
WBC_sysmex	0.77	0.00
PCV	0.72	0.00
TP	0.59	0.00

Table I.3: Summary of results for dimension 2. PCA on clinical signs associated with post-mortem visits.

	correlation	p.value
RT	0.82	0.00
TP	0.58	0.00
WBC_sysmex	-0.28	0.01
LymNum_sysmex	-0.38	0.00

Table I.4: Summary of results for dimension 3. PCA on clinical signs associated with post-mortem visits.

	correlation	p.value
RT	0.46	0.00
WBC_sysmex	0.42	0.00
PCV	-0.55	0.00

Table I.5: Summary of results for dimension 1. MCA on clinical signs associated with post-mortem visits.

	R2	p.value
RespiratoryProbs	0.41	0.00
Lethargy	0.34	0.00
OcularDischargeLac	0.33	0.00
Anorexia_DecApp	0.27	0.00
Cachexy_ExtremeThinness	0.26	0.00
Weakness	0.20	0.00
CornealOpacity	0.17	0.00
LNswelling	0.16	0.00
DeathCause	0.21	0.00
Froth	0.11	0.00
KidyInfarcts	0.11	0.00
LymIntInfHisto	0.11	0.01
KidneyGelatinisation	0.08	0.02
	Estimate	p.value
RespiratoryProbs_1	0.26	0.00
Lethargy_1	0.23	0.00
OcularDischargeLac_1	0.26	0.00
Anorexia_DecApp_1	0.22	0.00
Cachexy_ExtremeThinness_1	0.31	0.00
Weakness_1	0.19	0.00
CornealOpacity_1	0.39	0.00
LNswelling_1	0.15	0.00
LymIntInfHisto_1	0.17	0.00
Froth_1	0.12	0.00
KidyInfarcts_1	0.24	0.00
KidneyGelatinisation_1	0.11	0.02
KidneyGelatinisation_0	-0.11	0.02
other	-0.22	0.01
KidyInfarcts_0	-0.24	0.00
Froth_0	-0.12	0.00
LNswelling_0	-0.15	0.00
CornealOpacity_0	-0.39	0.00
Weakness_0	-0.19	0.00
Cachexy_ExtremeThinness_0	-0.31	0.00
Anorexia_DecApp_0	-0.22	0.00
OcularDischargeLac_0	-0.26	0.00
Lethargy_0	-0.23	0.00
RespiratoryProbs_0	-0.26	0.00

Table I.6: Summary of results for dimension 2. MCA on clinical signs associated with post-mortem visits.

	R2	p.value
WeightLoss_LossOfCondition	0.53	0.00
RoughStaringCoat	0.49	0.00
LNswelling	0.30	0.00
Weakness	0.20	0.00
KidneyGelatinisation	0.17	0.00
DeathCause	0.21	0.00
Froth	0.13	0.00
LymIntInfHisto	0.14	0.00
RespiratoryProbs	0.10	0.01
	Estimate	p.value
WeightLoss_LossOfCondition_1	0.27	0.00
RoughStaringCoat_1	0.24	0.00
LNswelling_0	0.19	0.00
Weakness_1	0.17	0.00
KidneyGelatinisation_1	0.14	0.00
Froth_0	0.12	0.00
Haemonchosis	0.27	0.00
LymIntInfHisto_0	0.16	0.00
RespiratoryProbs_0	0.12	0.01
LymIntInfHisto_1	-0.14	0.01
RespiratoryProbs_1	-0.12	0.01
Froth_1	-0.12	0.00
ECF	-0.20	0.00
KidneyGelatinisation_0	-0.14	0.00
Weakness_0	-0.17	0.00
LNswelling_1	-0.19	0.00
RoughStaringCoat_0	-0.24	0.00
WeightLoss_LossOfCondition_0	-0.27	0.00

	Estimate	p.value
Haemonchosis	-0.72	0.02

Table I.7: Summary of results for dimension 3. MCA on clinical signs associated with post-mortem visits.

	R2	p.value
Froth	0.24	0.00
KidneyGelatinisation	0.19	0.00
PericardialFluid	0.18	0.00
LymIntInfHisto	0.19	0.00
LNswelling	0.15	0.00
PethilHaem	0.12	0.00
DeathCause	0.17	0.00
WeightLoss_LossOfCondition	0.11	0.01
RoughStaringCoat	0.10	0.01
NervousSigns	0.09	0.01
icterusCS	0.09	0.01
Weakness	0.09	0.01
PeritonealFluid	0.07	0.02
CornealOpacity	0.06	0.04
Cachexy_ExtremeThinness	0.05	0.05
	Estimate	p.value
Froth_0	0.15	0.00
KidneyGelatinisation_0	0.14	0.00
PericardialFluid_0	0.15	0.00
LNswelling_0	0.12	0.00
PethilHaem_0	0.12	0.00
WeightLoss_LossOfCondition_0	0.11	0.01
RoughStaringCoat_0	0.10	0.01
NervousSigns_1	0.14	0.01
icterusCS_1	0.29	0.01
Weakness_1	0.10	0.01
PeritonealFluid_1	0.11	0.02
LymIntInfHisto.NA	0.11	0.03
CornealOpacity_1	0.19	0.04
Cachexy_ExtremeThinness_1	0.11	0.05
Cachexy_ExtremeThinness_0	-0.11	0.05
CornealOpacity_0	-0.19	0.04
PeritonealFluid_0	-0.11	0.02
Weakness_0	-0.10	0.01
icterusCS_0	-0.29	0.01
NervousSigns_0	-0.14	0.01
RoughStaringCoat_1	-0.10	0.01
WeightLoss_LossOfCondition_1	-0.11	0.01
PethilHaem_1	-0.12	0.00
LNswelling_1	-0.12	0.00
ECF	-0.20	0.00
PericardialFluid_1	-0.15	0.00
LymIntInfHisto_1	-0.18	0.00
KidneyGelatinisation_1	-0.14	0.00
Froth_1	-0.15	0.00

I.3 Data for analysis of decision support tool

Tables containing the diagnoses made by the DST, the scores associated with the different disease for each calf, and the clinical signs present in each calf.

Table I.8: Diagnoses made by the DST compared to the expert panel

CalfID	IDEAL_PrimaryCause	IDEAL_SecondaryCause	DST_primaryCause	DST_SecondaryCause
CA010110002	Haemonchosis		Trypanosomiasis	Schisto
CA010110012	East coast fever	Helminthisasis	Trypanosomiasis	PGE
CA010110019	Haemonchosis		Trypanosomiasis	Schisto
CA010110020	Haemonchosis	Theileriosis	Cowdriosis	PGE/Thei
CA010110021	Haemonchosis	Theileriosis	Cowdriosis	PGE
CA010110027	East coast fever	Rotavirus	Theileriosis	Cowdriosis
CA010210032	Unknown		Theileriosis	Cowdriosis
CA010210036	East coast fever		Theileriosis	Thei/Anaplas
CA010310064	Heartwater	East coast fever	Theileriosis	Cowdriosis
CA010310069	East coast fever	Haemonchosis	Trypanosomiasis	PGE
CA010310080	East coast fever		Cowdriosis	None
CA010310082	Haemonchosis	Dictyocaulus viviparous	Trypanosomiasis	None
CA020410093	East coast fever	Unknown	Theileriosis	Anaplasmosis
CA020510125	Haemonchosis	Helminthisasis	Cow/Fasc/Schist	None
CA020510142	East coast fever		Theileriosis	Cowdriosis
CA020610158	Unknown		Theileriosis	None
CA020610161	Unknown		Cowdriosis	None
CA020610172	East coast fever	Theileriosis	Theileriosis	None
CA030710187	Unknown	Unknown	Trypanosomiasis	Schisto
CA030710199	Unknown	Unknown	Cowdriosis	Tryps
CA030710200	Unknown	Helminthisasis	PGE	Anaplasmosis
CA030710201	East coast fever		Theileriosis	Trypanosomiasis
CA030710203	East coast fever	Helminthisasis	Theileriosis	Cowdriosis
CA030810211	Bacterial pneumonia	Unknown	Cowdriosis	None
CA030810217	Unknown	Unknown	PGE	None
CA030810234	Unknown	Unknown	Theileriosis	Cowdriosis
CA030910241	Unknown	Theileriosis	PGE	Anaplasmosis
CA030910247	Black Quarter	East coast fever	Theileriosis	Cowdriosis
CA030910254	East coast fever	Haemonchosis	PGE	Tryps
CA030910259	Unknown		Theileriosis	PGE
CA031010281	Haemonchosis	Helminthisasis	Trypanosomiasis	Theileriosis
CA031110308	East coast fever	Unknown	Trypanosomiasis	Cow/thei/schisto
CA031110314	East coast fever	Helminthisasis	Trypanosomiasis	Thei/PGE
CA031110325	Unknown		Cowdriosis	Theileriosis
CA031110326	Unknown	Helminthisasis	PGE	Tryps
CA031210335	East coast fever	Haemonchosis	Trypanosomiasis	Schisto
CA031210336	East coast fever		Trypanosomiasis	PGE
CA031210337	Actiomyces pyogenes	Unknown	Trypanosomiasis	Fasc

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Table I.8 – continued from previous page

CalfID	IDEAL_PrimaryCause	IDEAL_SecondaryCause	DST_primaryCause	DST_SecondaryCause
CA031210341	Unknown	Helminthisasis	PGE	Tryps/fasc/schisto
CA031210344	East coast fever	Unknown	Trypanosomiasis	PGE
CA031210350	East coast fever		Theileriosis	Cowdriosis
CA031310365	Heartwater		Trypanosomiasis	Fasc
CA031310367	Haemonchosis	Helminthisasis	Trypanosomiasis	Schisto
CA031310375	East coast fever		Theileriosis	Cowdriosis
CA031310381	Viral pneumonia	Unknown	Trypanosomiasis	Fasciolosis
CA031410399	Heartwater		Theileriosis	Cowdriosis
CA041510423	Unknown	Malignant catarrhal fever	None	None
CA041510435	East coast fever		Theileriosis	Cowdriosis
CA041610464	Unknown	Unknown	Theileriosis	Tryps/anaplas
CA041610465	East coast fever		Cowdriosis	None
CA041610468	Trypanosomiasis	Haemonchosis	Trypanosomiasis	Thei/PGE
CA041610470	Haemonchosis	Helminthisasis	Trypanosomiasis	PGE
CA041610477	Unknown	Unknown	Theileriosis	Tryps
CA041610478	East coast fever	Haemonchosis	Theileriosis	Tryps
CA041710487	Heartwater	Unknown	Theileriosis	Tryps
CA041710488	Turning sickness		Trypanosomiasis	PGE
CA041710501	Rabies		Cowdriosis	None
CA041710507	East coast fever		Theileriosis	None
CA051810535	East coast fever	Haemonchosis	Theileriosis	Tryps/anaplas
CA051910541	East coast fever	Trypanosomiasis	Trypanosomiasis	Schisto
CA051910556	Heartwater	Lead Poisoning	PGE	Theileriosis
CA051910557	East coast fever		Theileriosis	Cowdriosis
CA051910558	Arcanobacterium		PGE	Anaplasmosis
CA051910560	East coast fever		Theileriosis	None
CA052010571	Babesiosis		Cowdriosis	PGE/thei
CA052010576	East coast fever	poor nutrition	Theileriosis	None
CA052010577	East coast fever	Trypanosomiasis	Trypanosomiasis	Theileriosis
CA052010578	Heartwater		Theileriosis	Cowdriosis
CA052010580	East coast fever	Adenovirus	Theileriosis	Tryps/anaplas
CA052010581	Salmonellosis		Theileriosis	None
CA052010586	East coast fever		Trypanosomiasis	Theileriosis
CA052010587	East coast fever	Unknown	Trypanosomiasis	Thei/schisto
CA052010590	East coast fever	Unknown	Theileriosis	Anaplas/PGE/tryps
CA052010594	Unknown		Trypanosomiasis	Fasciolosis

Table I.9: Summary of the DST scores associated with each diagnosis

CalfID	AnaplasScore	Bab.Score	Cow.Score	PGE.Score	Thei.Score	Tryp.Score	Fasc.Score	Schisto.Score
CA010110002	9	7	7	11	8	15	11	12
CA010110012	6	4	0	10	1	11	8	9

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Table I.9 – continued from previous page

CalfID	AnaplasScore	Bab.Score	Cow.Score	PGE.Score	Thei.Score	Tryp.Score	Fasc.Score	Schisto.Score
CA010110019	8	5	7	11	7	13	11	12
CA010110020	2	2	4	3	3	0	1	1
CA010110021	6	4	8	8	4	7	5	6
CA010110027	5	6	8	3	11	3	1	1
CA010210032	3	3	7	4	7	4	4	4
CA010210036	7	6	4	6	9	7	3	5
CA010310064	0	0	4	0	4	2	0	0
CA010310069	4	2	0	6	5	6	3	5
CA010310080	3	3	7	1	3	2	3	3
CA010310082	4	2	0	5	5	9	4	5
CA020410093	9	8	8	6	15	7	3	5
CA020510125	4	3	7	6	3	6	7	7
CA020510142	5	6	8	3	14	3	1	1
CA020610158	2	2	4	0	10	2	0	0
CA020610161	3	3	7	4	3	2	4	4
CA020610172	6	4	4	6	11	6	3	5
CA030710187	8	5	11	11	4	13	11	12
CA030710199	3	2	8	4	3	7	5	4
CA030710200	6	4	4	6	4	4	3	5
CA030710201	9	8	8	5	12	10	4	5
CA030710203	2	2	4	3	7	2	1	1
CA030810211	3	3	11	1	3	2	3	3
CA030810217	0	0	0	3	0	0	1	1
CA030810234	2	2	4	0	6	0	0	0
CA030910241	6	4	4	6	4	4	3	5
CA030910247	5	6	8	0	14	3	0	0
CA030910254	4	2	0	8	1	7	5	6
CA030910259	0	0	0	3	4	2	1	1
CA031010281	3	2	4	4	7	9	5	4
CA031110308	8	5	11	8	11	15	10	11
CA031110314	6	4	4	8	8	9	5	6
CA031110325	5	6	8	5	7	4	3	2
CA031110326	0	0	0	5	0	3	3	2
CA031210335	8	5	7	11	11	15	11	12
CA031210336	7	4	4	10	8	13	8	9
CA031210337	1	0	0	4	0	7	5	4
CA031210341	1	0	0	5	0	4	4	4
CA031210344	7	4	4	10	4	11	8	9
CA031210350	5	6	8	0	14	3	0	0
CA031310365	4	3	7	5	6	9	8	7
CA031310367	8	5	7	6	4	10	8	10
CA031310375	2	2	4	0	10	2	0	0
CA031310381	4	3	7	5	3	9	8	7
CA031410399	3	2	8	5	10	6	4	4
CA041510423	0	0	0	0	0	0	0	0
CA041510435	5	6	8	0	14	3	0	0
CA041610464	6	4	4	3	8	6	2	4

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Table I.9 – continued from previous page

CalfID	AnaplasScore	Bab.Score	Cow.Score	PGE.Score	Thei.Score	Tryp.Score	Fasc.Score	Schisto.Score
CA041610465	3	3	7	1	3	2	3	3
CA041610468	3	2	4	7	7	9	6	5
CA041610470	3	2	4	7	3	7	6	5
CA041610477	10	8	8	10	15	14	8	9
CA041610478	10	8	8	5	15	11	5	7
CA041710487	2	2	4	2	7	5	2	1
CA041710488	3	2	4	7	3	7	6	5
CA041710501	2	2	8	0	3	0	0	0
CA041710507	2	2	4	0	10	2	0	0
CA051810535	6	4	4	3	8	6	2	4
CA051910541	8	5	7	6	8	12	8	10
CA051910556	3	3	11	4	10	4	4	4
CA051910557	9	8	12	3	15	7	2	4
CA051910558	6	4	4	6	4	4	3	5
CA051910560	2	2	4	3	10	2	1	1
CA052010571	2	2	4	3	3	0	1	1
CA052010576	2	2	4	3	7	2	1	1
CA052010577	11	9	11	11	15	16	11	12
CA052010578	3	3	7	1	10	4	3	3
CA052010580	7	6	4	6	9	7	3	5
CA052010581	2	2	4	3	7	2	1	1
CA052010586	3	2	4	4	7	9	5	4
CA052010587	8	5	7	9	11	12	9	11
CA052010590	6	4	4	6	8	6	3	5
CA052010594	2	1	3	8	0	9	9	8

Table I.10: Summary of clinical signs assessed as part of the DST - first set

CalfID	Anaemia	Anorexia_Depression	Ataxia_abonormalBehaviour	Diarrhoea	DyspnoeaCoughing
CA010110002	1	1	0	1	0
CA010110012	1	0	0	1	0
CA010110019	1	1	0	1	1
CA010110020	0	1	0	1	0
CA010110021	1	1	1	1	0
CA010110027	0	1	0	1	0
CA010210032	0	1	0	1	0
CA010210036	1	0	0	1	0
CA010310064	0	0	1	0	0
CA010310069	1	0	0	1	0
CA010310080	0	1	0	0	0
CA010310082	1	0	0	0	0
CA020410093	1	1	0	1	1

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Table I.10 – continued from previous page

CalfID	Anaemia	Anorexia_Depression	Ataxia_abonormalBehaviour	Diarrhoea	DyspnoeaCoughing
CA020510125	0	1	0	1	0
CA020510142	0	1	0	1	1
CA020610158	0	1	0	0	1
CA020610161	0	1	0	1	0
CA020610172	1	1	0	1	1
CA030710187	1	1	1	1	0
CA030710199	0	1	1	0	0
CA030710200	1	1	0	1	0
CA030710201	1	1	0	0	0
CA030710203	0	1	0	1	0
CA030810211	0	1	1	0	0
CA030810217	0	0	0	1	0
CA030810234	0	1	0	0	1
CA030910241	1	1	0	1	0
CA030910247	0	1	0	0	1
CA030910254	1	0	0	1	0
CA030910259	0	0	0	1	0
CA031010281	0	1	0	0	0
CA031110308	1	1	1	0	1
CA031110314	1	1	0	1	0
CA031110325	0	1	0	1	0
CA031110326	0	0	0	1	0
CA031210335	1	1	0	1	1
CA031210336	1	1	0	1	0
CA031210337	0	0	0	0	0
CA031210341	0	0	0	1	0
CA031210344	1	1	0	1	0
CA031210350	0	1	0	0	1
CA031310365	0	1	0	0	1
CA031310367	1	1	0	0	0
CA031310375	0	1	0	0	1
CA031310381	0	1	0	0	0
CA031410399	0	1	1	1	1
CA041510423	0	0	0	0	0
CA041510435	0	1	0	0	1
CA041610464	1	1	0	0	0
CA041610465	0	1	0	0	0
CA041610468	0	1	0	1	0
CA041610470	0	1	0	1	0
CA041610477	1	1	0	1	1
CA041610478	1	1	0	0	1
CA041710487	0	1	0	0	0
CA041710488	0	1	0	1	0
CA041710501	0	1	1	0	0
CA041710507	0	1	0	0	1
CA051810535	1	1	0	0	0
CA051910541	1	1	0	0	0

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Table I.10 – continued from previous page

CalfID	Anaemia	Anorexia_Depression	Ataxia_abonormalBehaviour	Diarrhoea	DyspnoeaCoughing
CA051910556	0	1	1	1	1
CA051910557	1	1	1	0	1
CA051910558	1	1	0	1	0
CA051910560	0	1	0	1	1
CA052010571	0	1	0	1	0
CA052010576	0	1	0	1	0
CA052010577	1	1	0	1	1
CA052010578	0	1	0	0	1
CA052010580	1	0	0	1	0
CA052010581	0	1	0	1	0
CA052010586	0	1	0	0	0
CA052010587	1	1	0	1	1
CA052010590	1	1	0	1	0
CA052010594	0	0	0	1	0

Table I.11: Summary of clinical signs assessed as part of the DST - second set

CalfID	Icterus	LymphNodeEnlargement	Pyrexia	RoughStaringCoat	Weakness	WeightLoss
CA010110002	1	1	0	1	1	1
CA010110012	1	0	0	1	0	1
CA010110019	0	0	0	1	1	1
CA010110020	0	0	0	0	0	0
CA010110021	0	0	0	1	0	0
CA010110027	0	1	1	0	0	0
CA010210032	0	1	0	0	1	0
CA010210036	0	1	1	0	0	0
CA010310064	0	1	0	0	0	0
CA010310069	0	1	0	0	0	0
CA010310080	0	0	0	0	1	0
CA010310082	0	1	0	1	0	0
CA020410093	0	1	1	0	0	0
CA020510125	0	0	0	0	1	1
CA020510142	0	1	1	0	0	0
CA020610158	0	1	0	0	0	0
CA020610161	0	0	0	0	1	0
CA020610172	0	1	0	0	0	0
CA030710187	0	0	0	1	1	1
CA030710199	0	0	0	1	0	1
CA030710200	0	0	0	0	0	0
CA030710201	0	1	1	1	0	0
CA030710203	0	1	0	0	0	0
CA030810211	0	0	0	0	1	0

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Table I.11 – continued from previous page

CalfID	Icterus	LymphNodeEnlargement	Pyrexia	RoughStaringCoat	Weakness	WeightLoss
CA030810217	0	0	0	0	0	0
CA030810234	0	0	0	0	0	0
CA030910241	0	0	0	0	0	0
CA030910247	0	1	1	0	0	0
CA030910254	0	0	0	1	0	0
CA030910259	0	1	0	0	0	0
CA031010281	0	1	0	1	0	1
CA031110308	0	1	0	1	1	1
CA031110314	0	1	0	1	0	0
CA031110325	0	0	1	1	0	0
CA031110326	0	0	0	1	0	0
CA031210335	0	1	0	1	1	1
CA031210336	0	1	0	1	0	1
CA031210337	0	0	0	1	0	1
CA031210341	0	0	0	0	0	1
CA031210344	0	0	0	1	0	1
CA031210350	0	1	1	0	0	0
CA031310365	0	0	0	1	1	1
CA031310367	0	0	0	0	1	1
CA031310375	0	1	0	0	0	0
CA031310381	0	0	0	1	1	1
CA031410399	0	1	0	0	0	1
CA041510423	0	0	0	0	0	0
CA041510435	0	1	1	0	0	0
CA041610464	0	1	0	0	0	0
CA041610465	0	0	0	0	1	0
CA041610468	0	1	0	1	0	1
CA041610470	0	0	0	1	0	1
CA041610477	0	1	1	1	0	1
CA041610478	0	1	1	0	0	1
CA041710487	0	1	0	1	0	0
CA041710488	0	0	0	1	0	1
CA041710501	0	0	0	0	0	0
CA041710507	0	1	0	0	0	0
CA051810535	0	1	0	0	0	0
CA051910541	0	1	0	0	1	1
CA051910556	0	1	0	0	1	0
CA051910557	0	1	1	0	0	0
CA051910558	0	0	0	0	0	0
CA051910560	0	1	0	0	0	0
CA052010571	0	0	0	0	0	0
CA052010576	0	1	0	0	0	0
CA052010577	0	1	1	1	1	1
CA052010578	0	1	0	0	1	0
CA052010580	0	1	1	0	0	0
CA052010581	0	1	0	0	0	0
CA052010586	0	1	0	1	0	1

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Table I.11 – continued from previous page

CalfID	Icterus	LymphNodeEnlargement	Pyrexia	RoughStaringCoat	Weakness	WeightLoss
CA052010587	0	1	0	0	1	1
CA052010590	0	1	0	0	0	0
CA052010594	0	0	0	1	1	1

Appendix J

Risk factors for ECF death

J.1 Univariable analysis summary for unmatched case control study

Table J.1: Summary of univariable analysis for unmatched case control study

Variables	Estimate	SE	P value	OR
AEZ 1	-	-	-	-
AEZ 2	-0.634	0.73	0.385	0.53
AEZ 3	-0.423	0.55	0.442	0.65
AEZ 4	-0.618	0.73	0.400	0.54
AEZ 5	0.136	0.59	0.817	1.15
SL 1	-	-	-	-
SL 2	-1.344	1.28	0.292	0.26
SL 3	-0.105	0.98	0.915	0.90
SL 4	-1.041	1.28	0.416	0.35
SL 5	-1.299	1.28	0.309	0.27
SL 6	-0.773	1.29	0.548	0.46
SL 7	-0.348	1.07	0.744	0.71
SL 8	-17.774	2776.67	0.995	0.00
SL 9	-17.774	2688.50	0.995	0.00

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Table J.1 – continued from previous page

Variables	Estimate	SE	P value	OR
SL 10	-17.774	2608.23	0.995	0.00
SL 11	-0.288	1.07	0.788	0.75
SL 12	0.539	0.95	0.571	1.71
SL 13	-0.981	1.28	0.445	0.37
SL 14	-17.774	2776.67	0.995	0.00
SL 15	-17.774	2688.50	0.995	0.00
SL 16	-0.348	1.07	0.744	0.71
SL 17	-1.099	1.28	0.391	0.33
SL 18	-1.299	1.28	0.309	0.27
SL 19	-0.460	1.07	0.666	0.63
SL 20	0.470	0.95	0.620	1.60
Elevation $<$ 1199m	-	-	-	-
Elevation $>$1269m	-0.013	0.50	0.979	0.99
Elevation 1199-1238m	-1.057	0.68	0.118	0.35
Elevation 1239-1269m	-0.257	0.51	0.617	0.77
Farmer sex male	-	-	-	-
Farmer sex female	0.017	0.42	0.968	1.02
Farmer age 10Y	0.410	0.15	0.005	1.51
Primary education TRUE	-	–	-	-
Primary education FALSE	-0.782	0.46	0.093	0.46
Professional training FALSE	-			1.00
Professional training TRUE	-0.137	0.48	0.774	0.87
No.adult female cattle	0.116	0.28	0.677	1.12
Feed supplementation FALSE	-			1.00
Feed supplementation TRUE	-0.463	0.44	0.293	0.63
Calf sex male	-			1.00
Calf sex female	-0.486	0.41	0.233	0.61
T. parva ELISA PP birth	0.002	0.01	0.815	1.00
T. parva ELISA $<$20PP	-			1.00
T. parva ELISA $>$20PP	-0.302	0.49	0.533	0.74
T. parva ELISA PP dam	0.015	0.01	0.047	1.02
T. parva ELISA dam $<$ 20PP	-			1.00
T. parva ELISA dam $>$ 20PP	0.641	0.48	0.177	1.90
European genetic introgression low	-			1.00
European genetic introgression high	0.102	0.54	0.849	1.11
Age infection T. parva 6:11 weeks	-			1.00

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Table J.1 – continued from previous page

Variables	Estimate	SE	P value	OR
Age infection T. parva 16:21 weeks	-1.003	0.49	0.041	0.37
Age infection T. parva 26:51 weeks	-1.905	0.52	0.000	0.15
T. mutans before T. parva FALSE	-			1.00
T. mutans before T. parva TRUE	-1.433	0.43	0.001	0.24
A. marginale before T. parva FALSE	-			1.00
A. marginale before T. parva TRUE	-1.493	1.04	0.150	0.22
B. bigemina before T. parva FALSE	-			1.00
fB. bigemina before T. parva TRUE	-1.163	1.03	0.261	0.31
Log(EPG) < 2	-			1.00
Log(EPG) 2-4	-1.381	1.13	0.220	0.25
Log(EPG) 4-6	-1.315	0.63	0.036	0.27
Log(EPG) 6-8	-0.621	0.58	0.284	0.54
log(EPG Haemochus) <2	-			1.00
log(EPG Haemochus) 2-4	-0.788	1.07	0.463	0.45
log(EPG Haemochus) 4-6	-0.585	0.53	0.268	0.56
log(EPG Haemochus) 6-8	-0.823	0.67	0.221	0.44
Grazing FALSE	-			1.00
Grazing TRUE	-2.297	0.62	0.000	0.10
Suckling FALSE	-			1.00
Suckling TRUE	0.723	1.05	0.492	2.06

J.2 Univariable analysis summary for matched case control study

J.2.1 Matched by age at *T. parva* infection (all available controls)

Table J.2: Summary of univariable analysis for age matched case control study containing 24 cases and X controls in 8 sets.

Variables	Estimate	SE	P value	OR
AEZ 1	REF			
AEZ 2	-1.00	0.37	-1.176	6.00
AEZ 3	-0.68	0.51	-1.156	7.00
AEZ 4	-0.71	0.49	-0.955	8.00
AEZ 5	-0.29	0.75	-0.470	9.00
SL 1	REF			
SL 2	-1.55	0.21	-1.201	35.00
SL 3	-0.53	0.59	-0.518	37.00
SL 4	-1.32	0.27	-1.011	38.00
SL 5	-20.68	0.00	-0.002	39.00
SL 6	-0.68	0.50	-0.523	40.00
SL 7	-0.77	0.46	-0.709	41.00
SL 8	-20.70	0.00	-0.001	42.00
SL 9	-20.70	0.00	-0.001	43.00
SL 10	-20.77	0.00	-0.001	25.00
SL 11	-0.64	0.52	-0.589	26.00
SL 12	-0.19	0.82	-0.191	27.00
SL 13	-20.69	0.00	-0.001	28.00
SL 14	-20.67	0.00	-0.001	29.00
SL 15	-20.70	0.00	-0.001	30.00
SL 16	-0.82	0.44	-0.740	31.00
SL 17	-1.45	0.24	-1.103	32.00
SL 18	-1.55	0.21	-1.192	33.00
SL 19	-1.00	0.37	-0.895	34.00
SL 20	-0.62	0.54	-0.603	36.00

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Table J.2 – continued from previous page

Variables	Estimate	SE	P value	OR
Elevation <1199 m	REF			
Elevation 1199-1238m	-0.93	0.39	-1.347	4.00
Elevation 1239-1269m	-0.04	0.97	-0.063	5.00
Elevation >1269m	0.06	1.06	0.105	3.00
Farmer sex male	REF			
Farmer sex female	0.28	1.32	0.625	17.00
Farmer age (10 years)	0.35	1.42	2.256	49.00
Primary education TRUE	REF			
Primary education FALSE	-0.60	0.55	-1.176	15.00
Professional training FALSE	REF			
Professional training TRUE	-0.34	0.71	-0.595	46.00
No.adult female cattle	-0.19	0.82	-0.622	50.00
Feed supplementation FALSE				
Feed supplementation TRUE	0.38	1.46	0.703	48.00
Calf sex male	REF			
Calf sex female	-0.39	0.68	-0.902	13.00
T. parva ELISA PP birth	0.00	1.00	0.529	1.00
T. parva ELISA <20PP	REF			
T. parva ELISA >20PP	-0.33	0.72	-0.616	12.00
T. parva ELISA PP dam	0.02	1.02	1.830	2.00
T. parva ELISA dam < 20PP	REF			
T. parva ELISA dam > 20PP	0.67	1.96	1.293	14.00
European genetic introgression low	REF			
European genetic introgression high	0.14	1.15	0.256	16.00
T. mutans before T. parva FALSE				
T. mutans before T. parva TRUE	-0.90	0.41	-1.863	45.00
A. marginale before T. parva FALSE	REF			
A. marginale before T. parva TRUE	-0.78	0.46	-0.720	10.00
B. bigemina before T. parva FALSE	REF			
B. bigemina before T. parva TRUE	-0.38	0.68	-0.358	11.00
Trypanosoma spp. before T. parva FALSE	REF			
Trypanosoma spp. before T. parva TRUE	-17.19	0.00	-0.003	47.00
Log(EPG) <2	REF			
Log(EPG) 2-4	-0.89	0.41	-0.799	22.00
Log(EPG) 4-6	-0.62	0.54	-0.913	23.00
Log(EPG) 6-8	-0.01	0.99	-0.018	24.00

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Table J.2 – continued from previous page

Variables	Estimate	SE	P value	OR
log(EPG Haemochus) <2	REF			
log(EPG Haemochus) 2-4	-0.08	0.93	-0.069	19.00
log(EPG Haemochus) 4-6	-0.09	0.92	-0.148	20.00
log(EPG Haemochus) 6-8	-0.65	0.52	-0.791	21.00
Grazing FALSE	REF			
Grazing TRUE	-2.40	0.09	-3.045	18.00
Suckling FALSE	REF			
Suckling TRUE	-0.17	0.84	-0.129	44.00

J.2.2 Matched by age at *T. parva* infection (one case to three controls)

Table J.3: Summary of univariable analysis for age matched case control study including 24 cases and 72 controls in 24 sets.

Variables	Estimate	SE	P value	OR
AEZ 1	REF			
AEZ 2	-1.45	0.23	-1.551	6.00
AEZ 3	-1.07	0.34	-1.583	7.00
AEZ 4	-0.94	0.39	-1.132	8.00
AEZ 5	-0.83	0.44	-1.147	9.00
SL 1	REF			
SL 2	1.50	4.48	0.716	35.00
SL 3	1.62	5.04	0.865	37.00
SL 4	-1.38	0.25	-0.735	38.00
SL 5	-21.87	0.00	-0.001	39.00
SL 6	2.77	16.04	1.202	40.00
SL 7	1.47	4.37	0.708	41.00
SL 8	-23.09	0.00	-0.000	42.00
SL 9	-21.60	0.00	-0.001	43.00
SL 10	-21.84	0.00	-0.001	25.00
SL 11	-1.26	0.28	-0.786	26.00
SL 12	2.58	13.18	1.338	27.00
SL 13	-21.23	0.00	-0.001	28.00
SL 14	-21.71	0.00	-0.001	29.00
SL 15	-21.72	0.00	-0.001	30.00
SL 16	1.43	4.20	0.742	31.00
SL 17	1.07	2.91	0.511	32.00
SL 18	-1.89	0.15	-0.995	33.00
SL 19	-2.27	0.10	-1.156	34.00
SL 20	0.54	1.71	0.341	36.00
Elevation <1199m	REF			
Elevation 1199-1238m	-0.81	0.44	-1.128	4.00
Elevation 1239-1269m	0.10	1.10	0.157	5.00
Elevation >1269m	0.81	2.26	1.197	3.00
Farmer sex male	REF			

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Table J.3 – continued from previous page

Variables	Estimate	SE	P value	OR
Farmer sex female	0.10	1.11	0.229	17.00
Farmer age (10 years)	0.26	1.29	1.599	49.00
Primary education TRUE	REF			
Primary education FALSE	-0.32	0.73	-0.576	15.00
Professional training FALSE	REF			
Professional training TRUE	-0.22	0.81	-0.349	46.00
No.adult female cattle	-0.06	0.94	-0.184	50.00
Feed supplementation FALSE	REF			
Feed supplementation TRUE	0.23	1.26	0.411	48.00
Calf sex male	REF			
Calf sex female	-0.48	0.62	-1.033	13.00
T. parva ELISA PP birth	0.01	1.01	0.918	1.00
T. parva ELISA <20PP	REF			
T. parva ELISA >20PP	-0.08	0.92	-0.143	12.00
T. parva ELISA PP dam	0.02	1.02	1.972	2.00
T. parva ELISA dam < 20PP	REF			
T. parva ELISA dam > 20PP	1.32	3.75	2.005	14.00
European genetic introgression low	REF			
European genetic introgression high	-0.09	0.92	-0.146	16.00
T. mutans before T. parva FALSE	REF			
T. mutans before T. parva TRUE	-0.69	0.50	-1.325	45.00
A. marginale before T. parva FALSE	REF			
A. marginale before T. parva TRUE	-0.38	0.69	-0.300	10.00
B. bigemina before T. parva FALSE	REF			
B. bigemina before T. parva TRUE	-0.00	1.00	-0.000	11.00
Trypanosoma spp. before T. parva FALSE	REF			
Trypanosoma spp. before T. parva TRUE	-18.47	0.00	-0.002	47.00
Log(EPG) <2	REF			
Log(EPG) 2-4	-1.47	0.23	-1.104	22.00
Log(EPG) 4-6	-1.03	0.36	-1.260	23.00
Log(EPG) 6-8	0.34	1.40	0.401	24.00
log(EPG Haemochus) <2	REF			
log(EPG Haemochus) 2-4	17.27	31605644.81	0.002	19.00
log(EPG Haemochus) 4-6	-0.40	0.67	-0.626	20.00
log(EPG Haemochus) 6-8	-0.60	0.55	-0.619	21.00
Grazing FALSE	REF			

Continued on next page

Table J.3 – continued from previous page

Variables	Estimate	SE	P value	OR
Grazing TRUE	-1.86	0.16	-2.410	18.00
Suckling FALSE	REF			
Suckling TRUE	-1.10	0.33	-0.777	44.00

J.3 Summary of models for the case control studies

These tables contain the model summaries discussed in the chapter, for the matched case control studies, both where all possible calves were used, and also when case control matched sets were formed.

Table J.4: Summary of conditional logistic regression model matched by age. The panel indicator used was the set identification number. The data set contained 24 cases and 333 controls in eight sets. Grazing was excluded to investigate the effect that missing data had on the association of *T. mutans* with death.

	Estimate	OR	LCL OR	UCL OR	P value
T. mutans before *T. parva* FALSE	-	-	-	-	-
T. mutans before *T. parva* TRUE	-1.557	0.211	0.064	0.690	0.010
European taurine genetic introgression FALSE	-	-	-	-	-
European taurine genetic introgression TRUE	-1.410	0.244	0.030	1.966	0.185
T. mutans before *T. parva* TRUE x European taurine genetic introgression TRUE	3.065	21.439	1.671	275.045	0.019

Table J.5: Summary of conditional logistic regression model matched by age. The panel indicator used was the set identification number. The data set contained 24 cases and 333 controls in eight sets. Grazing was excluded to investigate the effect that missing data had on the association of *T. mutans* with death.

	Estimate	OR	LCL OR	UCL OR	P value
T. mutans before *T. parva* FALSE	-	-	-	-	-
T. mutans before *T. parva* TRUE	-0.901	0.406	0.158	1.048	0.062

Table J.6: Summary of conditional logistic regression model matched age. The panel indicator used was the set identification number. Grazing was removed to examine the effect of missing data.

	Estimate	OR	LCL OR	UCL OR	P value
T. mutans before *T. parva* FALSE	-	-	-	-	-
T. mutans before *T. parva* TRUE	-1.181	0.307	0.093	1.008	0.052
European taurine genetic introgression FALSE	-	-	-	-	-
European taurine genetic introgression TRUE	-1.261	0.283	0.035	2.311	0.239
T. mutans before *T. parva* TRUE x European taurine genetic introgression TRUE	2.554	12.865	0.922	179.437	0.058

Table J.7: Summary of conditional logistic regression model matched age. The panel indicator used was the set identification number. The data contains 24 cases and 72 controls. The non-significant interaction was removed and it can be seen that the effect of *T. mutans* was no longer significant.

	Estimate	OR	OR_CI2.5	OR_CI97.5	P value
T. mutans before *T. parva* FALSE	-	-	-	-	-
T. mutans before *T. parva* TRUE	-0.758	0.469	0.158	1.388	0.171
Grazing FALSE	-	-	-	-	-
Grazing TRUE	-1.914	0.148	0.032	0.681	0.014

Table J.8: Summary of conditional logistic regression model matched age. The panel indicator used was the set identification number. The data contains 24 cases and 72 controls. Univariable model for prior exposure to *T. mutans*.

	Estimate	OR	LCL OR	UCL OR	P value
T. mutans before *T. parva* FALSE	-	-	-	-	-
T. mutans before *T. parva* TRUE	-0.691	0.501	0.180	1.393	0.185

J.4 Two by two tables for risk factors for ECF death

Table J.9: Two by two tables for the cases and controls included in the matched case control study with 24 cases and 333 controls with week of *T. parva* as the panel indicator. TMb4TP = Exposure to *T. mutans* prior to infection with *T. parva*. Gen int = whether the calf had >0.0156% European breed genetic introgression

	ECF dead FALSE	ECF dead TRUE
Grazing FALSE	134	21
Grazing TRUE	173	2

	ECF dead FALSE	ECF dead TRUE
TMb4TP FALSE	120	16
TMb4TP TRUE	213	8

gen int TRUE	ECF dead FALSE	ECF dead TRUE
TMb4TP	24	1
TMb4TP TRUE	33	4
gen int FALSE	ECF dead FALSE	ECF dead TRUE
TMb4TP FALSE	96	15
TMb4TP TRUE	176	4

Table J.10: Two by two tables for the cases and controls included in the matched case control study with 24 cases and 72 controls with set ID as the panel indicator. TMb4TP = Exposure to *T. mutans* prior to infection with *T. parva. Gen int = whether the calf had >0.0156% European breed genetic introgression*

	ECF dead FALSE	ECF dead TRUE
Grazing FALSE	37	21
Grazing TRUE	27	2

	ECF dead FALSE	ECF dead TRUE
TMb4TP FALSE	37	16
TMb4TP TRUE	35	8

gen int TRUE	ECF dead FALSE	ECF dead TRUE
TMb4TP FALSE	9	1
TMb4TP TRUE	7	4

gen int FALSE	ECF dead FALSE	ECF dead TRUE
TMb4TP FALSE	28	15
TMb4TP TRUE	28	4

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